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# THE EFFECTS OF DIFFERENT SALTS ON THE HEAT-PRODUCTION OF MUSCLE.

By E. SERENI (Rome).

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LITTLE attention has been paid to the effects, on the energy processes in muscle activity, of varying the chemical conditions of the medium. The fundamental work of A. V. Hill and others, on the differences resulting from the presence or absence of oxygen, is one important aspect of this problem: others are (a) the researches on the action of veratrine, from the earliest work of Fick(1) to the more recent work of Hartree and Hill(2); (b) the work of Weizsäcker(3) and quite recently of Gasser and Hartree(4) on the action of alcohol; and (c) the researches on the action of caffeine both on the heat-production(5) and on the chemical exchanges(6). These latter researches, however, deal with substances which are not naturally present in, or around, the muscle. Concerning the action of such substances as are to be found in the muscle itself, or in its surrounding medium—namely, the ions—there is little information. Apart from the recent paper by Hartree and Hill(7) on the effect of a change of hydrogen ion concentration, there are merely some researches by Weizsäcker(3) and by Gasser and Hartree(4) on the action of hypotonic solutions. In a short communication, Weizsäcker(8) states that increasing the amount of potassium produces the same effects as a hypotonic solution i.e. a parallel irreversible diminution both of heat-production and of tension—in his fuller report, however, no further information is given. Quite recently Embden(9) has published some researches on the action of different sodium salts on the lactic acidogen exchanges.

In an analogous field some interesting facts are to be found in a paper by Locke and Rosenbaum(10). These authors, working with the surviving mammalian (rabbit) cardiac muscle, succeeded in demonstrating that when circulating a Ringer's solution deprived of calcium and potassium (by which mechanical action is completely, or almost completely suppressed) the consumption of dextrose and the production of  $\text{CO}_2$  still go on though at a diminished rate. This diminution is mainly due to the lack of potassium the omission of which very greatly decreases

the dextrose consumption which is reduced but little by the removal of Ca.

In one of his earliest papers A. V. Hill<sup>(11)</sup> refers to some experiments conducted in NaCl instead of in Ringer; I shall deal later with these experiments, which, though planned from quite a different standpoint, bear a close relation to mine.

In my experiments the sartorius muscles of the frog (both *Rana temporaria* and *esculenta*) were used; in some of the experiments the animals were winter frogs from Holland and France, which had been kept in the tanks for some months; in the majority of the experiments the frogs were summer frogs and arrived in the laboratory only a few days before the experiment. Much depends upon the quality and breed of the frogs. Both males and females were used. The experiments were performed from July to September.

The general course of the experiments was as usual. A pair of sartorii were mounted on the thermopile (Fenn's type), placed in a glass-tube, and the tube sunk in a Dewar flask, filled with water, with air bubbling to keep the temperature uniform. The temperature in the Dewar flask varied from 13° C. to 16° C. in different experiments; during the course of each experiment it remained practically constant.

A Broca galvanometer was used for the measurements of the heat-production. This was surrounded by two cylindrical iron shields. The sensitivity of the galvanometer was about 1 mm. =  $10^{-10}$  ampère on a scale at a distance of three metres. In some experiments (the earliest) the sensitivity was less. The maximum deflection was reached in about 15 secs.

The muscles were tied to a platinum ring (one of the electrodes), soldered to an iron wire, which was attached to an isometric lever of the type described by Hill<sup>(12)</sup>.

The stimuli used were short tetani from a coil, the duration being determined by means of a Keith Lucas rotating drum. In each experiment, the muscle was stimulated six times for 0.1 sec. the successive stimuli decreasing in strength from supra-maximal to weak, then three times for 0.1 sec with stimuli either maximal or just sub-maximal, and finally three times for 0.5 sec. with the same stimuli. Each stimulation was separated from its neighbours by an interval of three minutes.

All the experiments were performed with the muscle immersed in an oxygenated solution, *i.e.* in Ringer, or variously altered Ringer. This has some advantages: *e.g.* the saving of time needed for the galvanometer to settle down after the solution has been withdrawn. On the other hand

the solution is still allowed to act between the stimulations; thus successive readings of the same "series" are not strictly comparable one to another as to their conditions. As, however, the readings for the different stimuli have always been performed in the same order, the readings for one and the same stimulus were always taken at the same time after the change of the solutions; so that it is safe to compare, one with another, the results for the same stimulus in different solutions. The amount of solution was always the same (200 cc.).

The first series (*i.e.* the series of 12 stimulations described above) was always taken in Ringer ( $\text{NaCl}$  0.63 p.c.,  $\text{KCl}$  0.03 p.c.,  $\text{CaCl}_2$  0.025 p.c.,  $\text{NaHCO}_3$  0.015 p.c.); a few preliminary stimuli were given, which seemed to make successive readings more regular. As soon as the series was finished, the solution was changed by means of a rubber tube running through the top of the muscle chamber, and 15 minutes was generally found sufficient for the galvanometer to settle down. At the end of this time, a new series of stimulations was completed; and so on, till the most altered solution was reached. When the series in this solution was finished, a return was made to the previous solution, and so on, in the reverse order, until Ringer was again reached. In these return series, however, owing to lack of time, the stimulations were limited to two of 0.1 sec. and two of 0.5 sec. with the moderately strong stimulus (see above); and often also to a single one of 0.1 sec. with the strongest stimulus. In some experiments the same stimulus, in strength and duration, was used throughout, to investigate the gradual changes occurring. At the end of the experiment the muscle was killed with chloroform and, some time after, controls performed with the same stimuli; the results of these, if any, were subtracted from the previous readings.

The following results are based mainly on the average of the three readings taken with the same moderately strong stimulus (of 0.1 sec. duration); the individual readings do not vary very much from one stimulus to another, and almost always only in a quantitative, and not in a qualitative sense. The reason why these particular readings were chosen rather than others, are (1) the fact that they are more reliable, because it is possible to take an average value, which diminishes the effect of occasional variations, (2) the fact that they were taken after the solution to be investigated had acted for rather a long time (33 mins.), and (3), what is still more important, after some previous contractions. In every case, reference will be made to the readings obtained with other stimuli where there has been any important difference.



*The effect of pure NaCl solution.* In a first group of experiments the effect of NaCl solution (0.7 p.c.) with the normal amount of  $\text{NaHCO}_3$  was tried. The muscle was first changed to a solution of 50 p.c. Ringer and 50 p.c. NaCl 0.7 p.c.; next to a solution of 25 p.c. Ringer and 75 p.c. NaCl 0.7 p.c.; and at the end to pure 0.7 p.c. NaCl. This means that the salts of the medium other than NaCl were reduced first to a half, afterwards to a quarter, and at the end were eliminated. In the reverse series the muscle was first returned to 25 p.c. Ringer—75 p.c. NaCl 0.7 p.c., afterwards to 50 p.c. Ringer—50 p.c. NaCl 0.7 p.c., and at the end to Ringer.

The effect of these changes on the height of the tension curves (Curve A, Figs. 1-3) is very distinct and sufficiently regular. The height

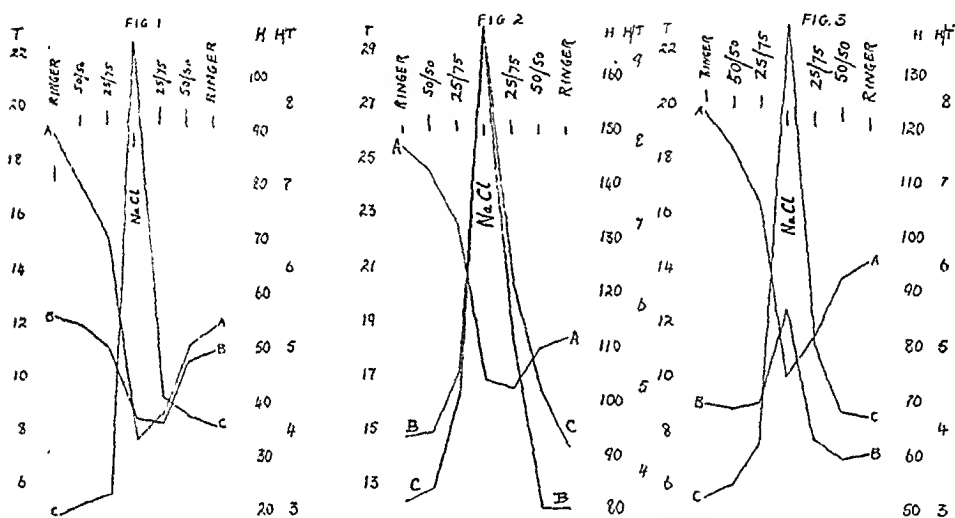


Fig. 1. Effect of NaCl solution on tension (A), heat-production (B), and heat/tension (C): mainly *Rana esculenta*. Average of several experiments.

Fig. 2. Effect of NaCl solution on tension (A), heat-production (B), and heat/tension (C): mainly *Rana temporaria*. Average of several experiments.

Fig. 3. Effect of NaCl solution on tension (A), heat-production (B), and heat/tension (C). Average of all experiments.

falls slowly in the solutions of 50 p.c. Ringer—50 p.c. NaCl and of 25 p.c. Ringer—75 p.c. NaCl; after that, in the pure NaCl solution, there is a sudden and more abrupt fall; and the height (in the reverse series) never attains its original value, at least within the time limits of these experiments. In some cases, when the muscle was changed back from pure NaCl solution to 25 p.c. Ringer—75 p.c. NaCl, the height of the tension curve did not rise, or only to an unimportant degree: indeed in some cases

there is a further decrease. This seems to point to the fact that sensitivity to the deprival of certain salts—or perhaps better, permeability to them—is subject to individual variations in the muscles. In some of them, an equilibrium is reached within the time limit of an experiment (50 mins. for each solution); in some it is not. Another explanation might be that salts other than NaCl, when reduced to  $\frac{1}{4}$  of the original concentration are unable to exert any action. Such a limit is well known for the action of NaCl on the excitability of muscle (13). Curve A, Fig. 3, represents the mean of 23 experiments, all yielding the same type of result with slight individual variations.

The change from Ringer to NaCl affects not only the height of the tension curve, but, as others have found before (e.g. Ringer (11), Locke (13)) also its shape. As these authors pointed out, this effect is rather variable and inconstant. Almost all these authors appear to have worked with *Rana temp.*, and it was found in the present experiments that in this frog the effect (which, in many features, recalls the action of veratrine) is much more easily produced than in *Rana esc.* This statement, however, has only a relative value: some *Rana temp.* have been found in which the effect of NaCl in giving a more or less distinct residual contracture was rather weak, while on the other hand some *Rana esc.* were used in which the effect was large. In a general sense it is possible to confirm almost completely the conclusions of previous authors. The effect is more evident with the stronger stimuli and sometimes appears only with these. An increase in the duration of the stimulus also favours the production of the effect: thus in some series contracture appears with the first strong stimulus, and then only with the last stimuli which are weaker but of longer ( $\frac{1}{2}$  sec.) duration. When a stimulus has produced a very intense prolongation of the response, the following one is likely to produce less, while the next will give a greater one again. This fact, which appears very clearly and without any doubt in the experiments in which the stimulus was kept constant, gives a suitable explanation of a phenomenon which would otherwise appear to be opposed to what has been said already about the action of the strength of the stimulus, this is, that sometimes, after a very intense contracture with a strong stimulus, the next or the two next stimulations, both with weaker stimuli, fail to give any contracture, or give only a small one—but then a third and still weaker stimulation gives again a more intense contracture.

When, after the action of NaCl, the muscle is brought back to Ringer, it recovers the normal shape of the tension curve. If we change it again

to NaCl, we often fail to see any change in the shape of the curve. This may be due to fatigue of the muscle: or we may accept the hypothesis of Cushing(26) who was the first to note this phenomenon, that  $\text{CaCl}_2$ , in these conditions, undergoes some more stable combination: and we know that it is  $\text{CaCl}_2$  which antagonises the action of NaCl.

Another phenomenon is that very often the residual contracture makes its appearance not only in pure NaCl solution, but also in the solution of 25 p.c. Ringer—75 p.c. NaCl 0.7 p.c., and even of 50 p.c. Ringer—50 p.c. NaCl. Individual differences may play an important part in the genesis of the phenomenon, and lowering of the concentration, without disappearance, of the salts other than NaCl may profoundly affect the muscles.

The effect of NaCl solution on the heat-production is also distinct (Curves B, Figs. 1-3). It appears that the effect may be of two alternative types. In the majority of cases (and they generally are of *Rana esc.*) the heat-production goes slowly down in 50 p.c. Ringer—50 p.c. NaCl and in 25 p.c. Ringer—75 p.c. NaCl: and falls more rapidly in pure NaCl solution, to rise again when changed back to Ringer. Curve B, Fig. 1, shows this type of result, mainly on *Rana esc.*, averaged for a number of experiments. As opposed, however, to what happens with the tension, the heat-production nearly recovers its original value or even sometimes surpasses it on return to Ringer. In some experiments, however (generally with *Rana temp.*), the course of the heat-production is completely reversed. Curve B, Fig. 2, shows the type of result on *Rana temp.*, also averaged over a number of experiments. After falling, or rising, more or less intensely in the first two solutions, in pure NaCl solution the heat-production suddenly increases, sometimes to very high values. All these are cases in which there was a very evident residual contracture: the reverse, however, is not true: all cases with a very evident contracture (and some of them with the most intense observed) do not present the rise of the heat-production.

NaCl solution has a considerable effect also on the ratio between heat-production and tension (Curves C, Figs. 1-3). In one sense this is the most important effect, because it is directly related to the "efficiency" of the muscle in producing tension. In a general sense, and excepting some few experiments, in NaCl solution the muscle is less efficient. This must obviously be the case when there is an appreciable rise of the heat-production and at the same time a diminution of the tension; it is no less true also for experiments in which the heat-production is diminished. This diminution is always proportionally less than

probably affects the mechanism which transforms chemical energy into mechanical. The second fact shows that, in the experiments in which a contracture appears, a part, and a large part, of the heat recorded in my experiments may be associated with the maintenance of a contracture; so that the apparent decrease in the "efficiency" of setting up a tension is less than that shown by the  $H/T$  ratio, since a large part of  $H$  is due to the prolongation of the contraction.

The ratio  $\frac{\text{heat rate during contracture}}{(\text{actual tension}) \times (\text{length})}$  is comparable (0.45) with that observed by Hartree and Hill(18) at the same temperature, in a tetanus and a veratrine contraction (about 0.60): the contracture in this case is maintained with slightly more than the usual economy.

An examination of the records obtained with the other strengths of stimulus gives practically the same results. The only thing worth noting is that, with the stronger stimuli or with the longer ones, the action of NaCl solution is often more intense. The rise of the  $H/T$  ratio is larger; and the heat-production rises in many cases in which with the moderately strong stimuli there was a decrease. This fact seems to indicate that the difference noted between *Rana temp.* and *esc.* is possibly only a difference of the stimuli needed to produce the effect.

The experiments recorded above cannot give completely satisfactory evidence about the time-course of the action of NaCl solution. For this purpose some experiments were performed in which the stimulus was kept constant and maximal, and repeated every three minutes. After some records in Ringer, the muscle was changed to pure NaCl solution, and records were taken as soon as the galvanometer was sufficiently settled (generally between 10 and 15 minutes). The results of these experiments were interesting. The tension went down with the well-known "irregularity" of muscles in NaCl solution. The heat-production in some cases goes down from the beginning, but very often it increases a certain amount at first, and then, if the experiment be continued for a sufficient time, diminishes again. Also in the course of the heat-production there are some "irregularities," probably due to the irregularity of response mentioned above (p. 5) which commonly follow strong contracture.

The heat/tension ratio rises steadily: and, strangely enough, its rise is not greatly affected by the irregularities of the tension and the heat-production. This is another argument for the hypothesis that the rise is due not only to the residual contracture, but also to some more intimate action of NaCl. In almost every case, in the first or in the two first records after the change to NaCl solution, the  $H/T$  ratio, instead of

the diminution of the tension. Naturally, the rise in the ratio between heat-production and tension is larger in the former experiments than in the latter but with few exceptions the rise is always quite evident. When the muscle is brought back to Ringer, the H/T ratio diminishes again and within the time limits of these experiments, it sometimes reaches or even falls below the initial value. Sometimes the maximum is not reached in NaCl, but in 25 p.c. Ringer—75 p.c. NaCl solution. This is clearly related to the similar fact in the case of the tension. A second change to NaCl solution often does not affect (or only slightly) the H/T ratio, and this fact, when compared with the parallel failure of NaCl solution in these experiments to produce a residual contracture, seems to indicate that the rise in the H/T ratio is intimately related to the production of the residual contracture. On the other hand, there are some experiments in which the H/T ratio rises without any, or only little, appearance of residual contracture pointing to an action of NaCl on the mechanism itself which transforms the chemical energy into the mechanical one.

In order to decide upon these points it was necessary to analyse the time course of the heat-production after the action of NaCl solution. Mr W. Hartree kindly undertook this analysis in the physiological laboratory at Cambridge and I wish to express to him my best thanks for his kindness in allowing me to publish his results. In his experiments the conditions were slightly different from those in mine. After dissection (using Ringer) the muscle was brought directly to NaCl solution (with the normal amount of  $\text{NaHCO}_3$ ), which was allowed to act for about one hour then the solution was withdrawn and the records (stimuli 0.1 and 0.2 sec.) were taken as soon as possible. Strong stimuli were used for the records (12.5 volts). The muscle was then left for three hours in Ringer which was then removed and 'normal' contractions were taken afterwards controls were made as usual (7). The results after NaCl were corrected for the heating effect of the stimulating current, which was comparatively large (17 p.c. of the whole). The tension curve after NaCl presents a distinct residual contracture. The analysis of the heat production shows that, under the effect of NaCl the heat developed in the first half second is about 30 p.c. greater than in the normal muscle (although the maximum tension is 8 p.c. less), and in the subsequent prolonged relaxation an amount of heat appears equal to about 4 times the normal initial heat production. The first fact confirms what was assumed from the experiments which produced an increase of the H/T ratio, without any contracture, i.e. that the NaCl

probably affects the mechanism which transforms chemical into mechanical. The second fact shows that, in the experiment in which a contracture appears, a part, and a large part, of the heat of my experiments may be associated with the maintenance of the contracture; so that the apparent decrease in the "efficiency" of the heat-tension is less than that shown by the H/T ratio, since a part of the H is due to the prolongation of the contraction.

The ratio  $\frac{\text{heat rate during contracture}}{(\text{actual tension}) \times (\text{length})}$  is comparable (0.4) to that observed by Hartree and Hill (18) at the same temperature in tetanus and a veratrine contraction (about 0.60): the contracture case is maintained with slightly more than the usual economy.

An examination of the records obtained with the other method of stimulus gives practically the same results. The only difference to be noted is that, with the stronger stimuli or with the longer duration of action of NaCl solution is often more intense. The rise of the heat-tension is larger; and the heat-production rises in many cases in which with moderately strong stimuli there was a decrease. This fact indicates that the difference noted between *Rana temp.* and *esc.* is due to only a difference of the stimuli needed to produce the effect.

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The heat/tension ratio rises steadily: and, strangely enough, it is not greatly affected by the irregularities of the tension and the heat-production. This is another argument for the hypothesis that the heat-tension ratio is due not only to the residual contracture, but also to some more in the action of NaCl. In almost every case, in the first or in the two or three records after the change to NaCl solution, the H/T ratio, instead

is, though rarely, the appearance of a residual contracture. Previous authors are of different opinions about the action of KCl on the residual contracture produced by pure NaCl. According to Ringer<sup>(13, 14)</sup> KCl increases the effect; according to Fahr<sup>(19)</sup> and Mines<sup>(20)</sup> it abolishes it.

The heat-production also is much impaired by the deprival of  $\text{CaCl}_2$  (Curve B, Fig. 5). It quickly decreases in the solution with a diminished amount of  $\text{CaCl}_2$ , and rises again as soon as  $\text{CaCl}_2$  is increased: indeed it sometimes reaches the initial value again.

The ratio between heat-production and tension is diminished (Curve C, Fig. 4). The diminution and the increase which follow when  $\text{CaCl}_2$  is respectively reduced and restored proceed almost at the same rate, and the final value not only reaches but often exceeds the initial value. This means that in this case also, when coming back to normal conditions of salt-content, the recovery is at first stronger and quicker in the chemical processes.

The decrease of the H/T ratio occurred in all but two experiments (*Rana temp.*) and in these there was a prolonged relaxation. In one of them there was practically no change: in the other there was quite a notable rise. A second transference to Ca-free Ringer often leaves the H/T ratio unaffected; in this case also there seems to have been some irreversible change.

Some experiments were made with a regular series of constant stimuli. The tension comes down, more rapidly than in the NaCl experiments; the heat-production comes down also, excepting when there is a residual contracture, which is accompanied by a rise, sometimes large. In these cases there is also a rise of the H/T ratio; in the other cases there is a progressive decrease of the ratio. It is interesting to note that in this case also, in the first records after the change from Ringer to Ca-free Ringer, the change of the H/T ratio is very often opposite to the change we observe later—there is a rise instead of a fall.

*The effect of K-free Ringer.* The solution used in these experiments contained NaCl 0.66 p.e.,  $\text{CaCl}_2$  0.025 p.e.,  $\text{NaHCO}_3$  0.015 p.e. The change from Ringer to this solution was as usual gradual; the intermediate solutions were like those in the former experiments. The effect on the tension height (Curve A, Fig. 5) is to diminish it, but the diminution is less abrupt than in the case of NaCl solution or Ca-free Ringer. The fall is much more gradual, without any sudden change, and, what is still more important, when the muscle is brought back to Ringer, the recovery is quite small, or often absent altogether, and the tension still goes down. The deprival of KCl, if it affects the muscle much more slowly and

apparently less intensely, does it in an irreversible manner, at least within the time limits of these experiments. The deprival of KCl does not affect in any way the shape of the tension-curves.

The heat-production also goes down (Curve B, Fig. 8). As in the case of the tension-height, the decrease is much more gradual than in NaCl solution or Ca-free Ringer, and the recovery is small: indeed in some experiments the heat-production decreases steadily until the end, and the lowest value is attained in the final immersion in Ringer.

With these much less distinct alterations in the tension and in the heat-production, it was inevitable that the changes in the H/T ratio should also be less obvious (Curve C, Fig. 5). On the whole there is a slight tendency for the H/T ratio to decrease, as an effect of the K-free Ringer. After the minimum has been reached in completely K-free Ringer, the course of the H/T ratio varies greatly. In some cases there is a regular rise; in some first a rise, then a fall. Sometimes there is no rise at all, and the ratio goes on falling. On the average there is a rise. No satisfactory explanation can be offered of these differences.

An interesting fact is that, as opposed to what happens in the experiments with NaCl solution and Ca-free Ringer, the second contraction with the same stimulation, in the return-series, gives almost invariably less heat-production and less tension than the first one.

The experiments in which the stimulation was kept constant throughout gave practically the same results. Heat-production and tension ran down rather slowly, and there was also a distinct though small decrease of the ratio between them. An increase of the ratio in the first contractions after the change of the solution is often evident, and it lasts longer than in the cases previously described. Sometimes this initial increase is rather large, and the later decrease only brings the ratio back to its initial value, or a little below.

In some experiments the action was tested of K-free, followed by Ca-free Ringer. The decrease of both heat-production and tension is steady and becomes more rapid when the muscle is changed from the K-free to the Ca-free solution. Also the ratio between heat-production and tension, which diminishes only a little in the K-free Ringer, shows a further decrease in the Ca-free Ringer.

*The action of Ringer with an increased amount of K.* The stock solutions for an increased K concentration were the usual Ringer and a modified Ringer containing four times the normal amount of KCl and (for isotonic reasons) rather less NaCl (0.55 p.c.). These solutions were mixed in the same proportions as in the other experiments. The muscles



became inexcitable in 25 p.e. Ringer and 75 p.e. modified Ringer and sometimes in 50 p.e. Ringer + 50 p.e. modified Ringer.

The effect on the tension is striking (Curve A, Fig. 6). The tension-height falls very quickly. It reaches zero in the 25 p.e.—75 p.e. solution,

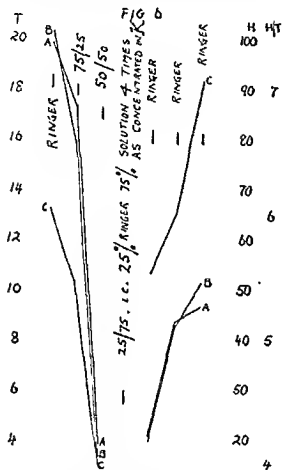
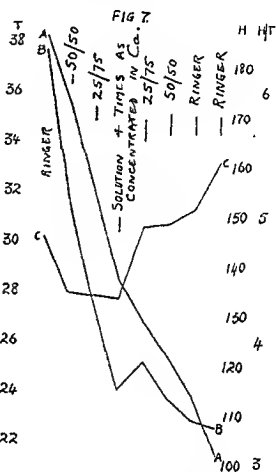


Fig. 6. Effect of an increased proportion of K on tension (A), heat-production (B), and heat/tension (C). Average of 9 experiments.

Fig. 7. Effect of an increased proportion of Ca on tension (A), heat-production (B), and heat/tension (C). Average of 10 experiments.



or, sometimes, even in the 50 p.e.—50 p.e. solution; and it remains at this point when the muscle is brought back to the less concentrated solutions. If the immersion in the 75 p.e. Ringer—25 p.e. modified Ringer solution be prolonged, there is a slight rise in the tension; when the muscle is brought back to the Ringer solution the rise is at first very rapid: after some time it becomes less. A further rise can be observed for a long time after the muscle has been changed back to Ringer. Sometimes, after a longer immersion in Ringer, the tension decreases a little as if, in addition to paralysing the muscle, the excess of KCl had a poisoning effect.

The effect on the heat-production is similar to that on the tension (Curve B, Fig. 6). Here also the zero line is reached in the 50 p.e.—50 p.e. or in the 25 p.e.—75 p.e. solution; and there is only a small

amount of recovery after a longer immersion in 75 p.c. Ringer—25 p.c. modified Ringer. The heat-production, just as the tension, rises again only when the muscle is brought back to Ringer. It is interesting to note that the rise of the heat-production is quicker than that of the tension. In this case also it seems as if the chemical processes reappear first, and only afterwards the possibility of their transformation into mechanical processes. The heat-production, as well as the tension, after a longer immersion in Ringer, sometimes tends to decrease slightly.

The changes in the H/T ratio are very large (Curve C, Fig. 6). The effect is the same as in the case of the Ca-free Ringer, that is a decrease, but larger. The ratio decreases rapidly in the 75 p.c.—25 p.c. and 50 p.c.—50 p.c. solutions, and rises again, but less rapidly, when the muscles are brought back to Ringer. The recovery of both tension-height and heat-production which takes place in Ringer continues with a steady increase of the ratio.

It is interesting to note how the recovery proceeds in Ringer. By comparing the two records with the same stimulus in the same series and in different series, it appears that the stimulation itself greatly quickens the recovery process. The increase in the tension and in the heat-production between the first and the second record of every series (that is, in three minutes) is often only slightly less than the changes from the second record to the first of the next series (that is, in 21 minutes). This effect is more evident for the tension than for the heat-production.

In some experiments the muscle was changed directly from Ringer to 25 p.c.—75 p.c. or 50 p.c.—50 p.c., or 75 p.c.—25 p.c. solution and a regular series of maximal stimuli applied. As soon as the muscle had become inexcitable for the stimulus given, it was changed back to Ringer. Readings were taken as soon as possible. Inexcitability is reached after about 30 minutes in the 25 p.c. Ringer—75 p.c. modified Ringer solution, only after about 60 minutes in the 50 p.c.—50 p.c. solution. The heat/tension ratio falls from the first contraction in the 25 p.c.—75 p.c. solution; in the 50 p.c.—50 p.c. solution it remains almost constant, with a tendency to decrease, for some contractions; then it begins to fall more rapidly. As soon as the tension and the heat-production rise after restoration to the normal Ringer's solution, the H/T ratio begins to rise steadily, till it reaches, and sometimes surpasses, its initial value. At the end there is a tendency of the H/T ratio to fall a little.

The recovery takes more time than the paralysis, and the degree of

recovery depends not so much on the amount of KCl as on the time this salt had been allowed to act. For instance, there has been a more complete recovery after complete inexcitability produced by 25 p.c.—75 p.c. solution than after complete inexcitability produced by 50 p.c.—50 p.c. solution: presumably because the first, having a more rapid action, was allowed to act on the muscle for a shorter time. After the muscle has recovered in Ringer, a second change to the modified Ringer reproduces the same effect.

It is also interesting to observe that in no case was there any sign of residual contracture. The presence of a normal amount of  $\text{CaCl}_2$  was apparently sufficient to check the tendency of NaCl to give the contracture, which KCl alone cannot always antagonise, as shown in the experiments with Ca-free Ringer.

*The action of Ringer with an increased amount of  $\text{CaCl}_2$ .* The modified solution contained four times the normal amount of  $\text{CaCl}_2$ . Solutions with different increased amounts of Ca were prepared and used in the way described above.

The tension-height continually diminishes from the beginning to the end of the experiment (Curve A, Fig. 7). The decrease is generally more rapid in the first changes until the completely modified Ringer is reached; then it becomes slower, but becomes more rapid again during the prolonged final immersion in Ringer. In one case only was there a little recovery: in this, after returning to Ringer and after a first series of records had been obtained, the muscle was left unstimulated for more than two hours, before the observations in question.

The heat-production in a general sense presents the same decrease from the beginning to the end of the experiment (Curve B, Fig. 7); this decrease is less regular than was that of the tension, and there are some variations during its course. So, for instance, there is often a little rise in the heat-production when the muscle is brought back from the completely modified solution to the 25 p.c.—75 p.c. solution, as if there were the beginning of a recovery-process. Very soon after this rise the decrease begins again, and with some little irregularities it continues to the end. An interesting feature is that, as in the case of KCl-free Ringer, but much more obviously, the second contraction in the return-series with the same stimulus almost invariably gives less tension and much less heat than the first one. This effect is so distinct that, when at the end of each of the return-series (this means, after four moderately strong stimuli, two of  $\frac{1}{10}$  sec. and two of  $\frac{1}{2}$  sec.) the muscle was stimulated with the strongest stimulus ( $\frac{1}{10}$  sec. duration), the record for both tension and

heat-production was often less than in the two  $\frac{1}{10}$  sec. stimulations with the moderately strong stimulus. In the same case in which, after a longer immersion in Ringer, without any stimulation, there was a rise in the tension, there was a larger increase in the heat-production. The rise in the heat-production in the first stages of the return-series can be perhaps assumed to indicate that  $\text{CaCl}_2$  has a double action, first an inhibition, then a poisonous effect.

The H/T ratio presents an initial decrease (Curve C, Fig. 7), followed by a rise, which is rather irregular in its course, and reaches values beyond those at the beginning. The decrease sometimes appears only in the first change, sometimes there is a further decrease in the 25 p.c.—75 p.c. solution and in the completely modified Ringer. In no case, however, is there a further decrease in the next changes: the rise is generally more rapid than the descent, and it reaches high values, which means a low "efficiency" of the muscle.

### *Discussion.*

The experiments described above show, that by changing the saline content of the medium it is possible to affect profoundly not only the tension, but also the heat-production of the muscle. That the changes in this latter are not merely due to the changes in the former is proved by the variations in the H/T ratio; these show that the variations of the saline content affect the "efficiency" of the muscle in developing tension, which is apparently not at its highest point with the "normal" proportions of the different salts.

Before we attempt to explain these phenomena, it is advisable to ascertain if there is any possibility of attributing them to purely technical factors. It was first pointed out by Weizsäcker(3) that, in such experiments as these, it is possible that the heat-production recorded is mainly due to the superficial layer of the muscle, *i.e.* to the layer on which any alteration of the medium is allowed first to act. It is possible, therefore, that the heat-production may appear to be altered at a moment when the tension (which is the effect of all the fibres) is still nearly unchanged. The same considerations might also give a simple explanation of the fact recorded above, that the heat-production, in the return-series, appears to be restored before the tension. It is improbable that this possibility is of any great importance when working with such a slow-moving galvanometer as that used: the temperature of a thin sartorius muscle should be fairly well equalised in 15 secs. If, moreover, the effects recorded were only, or mainly, due to this simple fact it would be im-

possible to explain why there is (after restoration to the normal solution) a gradual rise of the H/T ratio after the former decrease. As the superficial fibres are restored first, so there must be a moment when only these fibres are restored, and both heat-production and tension are due only to them; at this very moment the H/T ratio must be at its maximum, to descend afterwards, when the more remote fibres are restored, the tension of which is recorded but not the heat-production. This is not so, and can be safely assumed that, whatever its importance may be, this possibility cannot give a full explanation of the whole of the results.

The experiments of A. V. Hill(40) on the effect of NaCl were made with the purpose of showing that "tone" produces less heat than "contraction." By giving a stimulating current of three shocks per second, he found that the "tonus" set up in NaCl solution was greater than in Ringer, and the heat-production less. These results are not comparable with mine. In the same paper, however, Hill noted that sometimes (and more often with weaker stimuli in NaCl solution) the muscle shows a prolonged heat-production, accompanied by a contracture. The effect is weakened when many stimulations are given; but it reappears after a long rest. All these facts are in perfect accord with the results of my experiments with NaCl solution.

Summing up the results of my experiments, it has been shown that the different ions not only regulate the processes of excitability of muscle, but also modify the utilisation of the energy which the stimulation sets free. Varying the proportions of different salts influences the working conditions of the muscles. Considering the results of the variations, we see that all the changes diminish the absolute value of the response of the muscle to a constant stimulus, both as regards the tension and heat-production (excepting, as regards the last, some frogs (usually *temporaria*) in the NaCl experiments).

On the other hand, important variations are observed in the H/T ratio. The "efficiency" of the muscle is decreased (i.e. the H/T ratio is increased) by pure NaCl: but the "efficiency" is increased by excess of KCl (Ringer without  $\text{CaCl}_2$  or with increased KCl) and to a less extent by excess of  $\text{CaCl}_2$  (Ringer without KCl or with increased  $\text{CaCl}_2$ ).

It is strange that the effects of excess of KCl and of excess of  $\text{CaCl}_2$  are both in the direction of a decrease of the H/T ratio. It is true that the effect is more considerable for KCl than for  $\text{CaCl}_2$ , but it is often possible to demonstrate a distinct effect also for  $\text{CaCl}_2$ . Therefore as regards the action on the energy processes in muscle there is no anta-

indicates but a small amount of  $O_2$  in solution, so that we have further proof that there is no great storage of  $O_2$  therein.

In my previous papers(4) I have studied certain experimental changes in the  $CO_2$ - and  $O_2$ -tensions under the skin and in the abdominal cavity. In the present paper the  $O_2$ -tension in the inspired air has been varied and the effects upon the  $O_2$ -tension in the tissues are considered.

*Method.* The technique for injection of gas under the skin and into the abdominal cavity and for withdrawal of samples therefrom has been fully described(4). The  $O_2$ -tension in the inspired air was varied from 11 to 90.77 p.e., gas mixtures being made up—from air and  $N_2$  or  $O_2$ —in a balloon capable of holding 2500 litres. The exact composition of the mixture was accurately obtained by duplicate analyses in the Haldane apparatus; with high  $O_2$ -content only about 2 c.c. were analysed by mixture with nitrogen in the usual way. The temperature of the gas breathed was that of the room, namely 16–18° C.; a thermometer was inserted into the balloon.

The animal (rabbit) was not anaesthetised and breathed the gas through the small mask illustrated diagrammatically in Fig. 1<sup>1</sup>. The mask was fitted with delicate valves designed by Lovatt Evans.

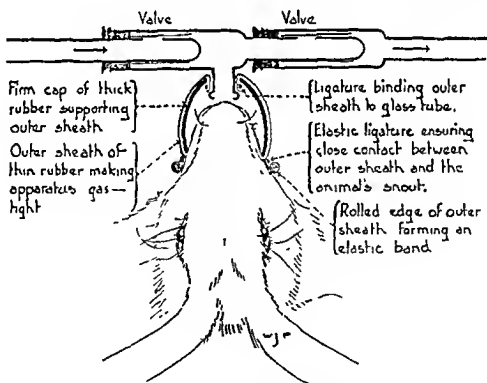


Fig. 1. For description, see text.

With gentle handling the animal remained quite still in a sleepy condition on its back for six hours. It was placed on a thick pad of cotton-wool on a table and fixed in the usual manner but pressure points

<sup>1</sup> I am indebted to Dr W. J. Purdy for this figure.

# THE INFLUENCE OF $O_2$ -TENSION IN THE INSPIRED AIR UPON THE $O_2$ -TENSION IN THE TISSUES.

By J. ARGYLL CAMPBELL.

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It is still generally believed that the  $O_2$ -tension in the tissues is nil although Ehrlich's method(1) upon which this belief is founded has been proved to be untrustworthy. He used certain stains which have now been proved to be reduced even if free molecular oxygen is present. The earlier estimations of oxygen in secretions—which were said to contain little or no oxygen—were also at fault because oxygen is used up in these secretions. Krogh(2) has found that the tension of oxygen in urine is not nil if determined in freshly voided urine but is as much as 35 mm. Hg. Verzár(3) proved by an ingenious method that the normal oxygen tension in the salivary glands is about 44 mm. whilst in voluntary muscle it is about 20 mm. He did not prove that the tension in the tissues was nil, as is sometimes stated.

During the past two years I(4) have determined the  $O_2$ -tension in gas injected under the skin and into the abdominal cavity of rabbits and cats and have found, as the result of about 300 observations, that the normal  $O_2$ -tension under the skin is about 20–30 mm. Hg and in the abdominal cavity about 30–40 mm. Hg, the tensions for any one animal remaining remarkably constant over long periods of time. These tensions may be regarded as the tensions present in the fluids which exist immediately outside and bathe the cells of the subcutaneous tissue and the cells of the peritoneum of the abdominal cavity. The higher  $O_2$ -tension in the abdominal cavity is probably due to better circulation there than under the skin. My method of injection of gas therefore on theoretical grounds should supply similar information as regards  $O_2$ -tension in the tissues as the methods of Krogh and Verzár mentioned above. It must be admitted that none of these methods estimates directly the  $O_2$ -tension inside the cell but they go one step further than do analyses of gases of the blood, in that they give the conditions in the fluid which lies outside the capillaries and which is in contact with the wall of the cell. The presence of this tension in the tissue fluids

indicates but a small amount of  $O_2$  in solution, so that we have further proof that there is no great storage of  $O_2$  therein.

In my previous papers (4) I have studied certain experimental changes in the  $CO_2$ - and  $O_2$ -tensions under the skin and in the abdominal cavity. In the present paper the  $O_2$ -tension in the inspired air has been varied and the effects upon the  $O_2$ -tension in the tissues are considered.

*Method.* The technique for injection of gas under the skin and into the abdominal cavity and for withdrawal of samples therefrom has been fully described (4). The  $O_2$ -tension in the inspired air was varied from 11 to 90.77 p.c., gas mixtures being made up—from air and  $N_2$  or  $O_2$ —in a balloon capable of holding 2500 litres. The exact composition of the mixture was accurately obtained by duplicate analyses in the Haldane apparatus; with high  $O_2$ -content only about 2 c.c. were analysed by mixture with nitrogen in the usual way. The temperature of the gas breathed was that of the room, namely 16–18° C.; a thermometer was inserted into the balloon.

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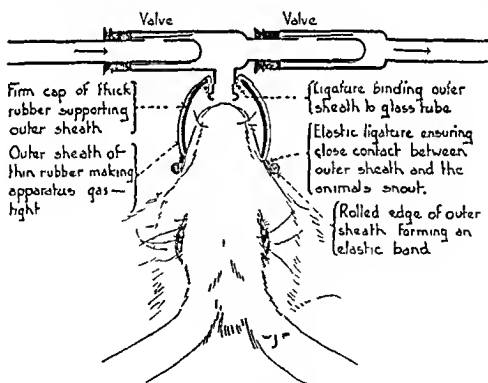


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With gentle handling the animal remained quite still in a sleepy condition on its back for six hours. It was placed on a thick pad of cotton-wool on a table and fixed in the usual manner but pressure points

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were well padded with cotton-wool. The animal's head, except its snout, was also swathed in cotton-wool and the eyes were covered. The head was then fixed in an upright position by means of a suitable clamp, the back of the head resting on a soft pad on the table. The animal was not tightly fixed and was able to move, but only to a small extent, in any direction. In some cases vaseline was smeared over the snout where ligatures were applied.

The mask consisted of a firm cap of thick rubber which enclosed the snout without touching the nostrils. This cap was simply an old oval syringe bulb cut down to suitable shape and size. The mask was made air-tight by an outer cylindrical sheath of thin but strong rubber, narrowed at one end so as to fit on the short arm of a T-shaped glass tube which was held in position by a clamp; the horizontal portion of the glass tube carried the inlet and outlet valves. The figure shows in detail how the apparatus was made air-tight. There was no slack rubber at any point except at the valves so that the efforts of the respiratory muscles were exerted only to open and to close these delicate valves of guinea-pig's peritoneum.

*Breathing air (i.e.  $O_2 = 20.93$  p.c.) through the mask.* As a control it was necessary, first of all, to determine the effects—if any—of breathing ordinary air through such an apparatus upon the  $CO_2$ - and  $O_2$ -tensions under the skin and in the abdominal cavity. At the same time, as a further control, I estimated the  $CO_2$ -output and the  $O_2$ -intake, by the Douglas-Haldane method, to obtain details of the respiratory exchange, the animal breathing into a small Douglas bag for about 15 minutes.

The results of a typical experiment are given in Table I. It will be observed that breathing air through the apparatus for 150 minutes produced practically no effect upon the  $O_2$ -tensions under the skin and in the abdominal cavity; the tension did fall about 1 mm. but this amount spread out over 150 minutes may be neglected. I give also in Table I the effects upon the  $CO_2$ -tension in these regions. It will be noted that the  $CO_2$ -tension increased from 47 to 52 mm. under the skin and from 45 to 52 mm. in the abdominal cavity; this increase was probably due to the effect of breathing against the resistance of the valves which was  $\frac{1}{2}$  cm.  $H_2O$ . Haldane(5) has shown that breathing against a resistance causes an increase in alveolar  $CO_2$ -tension since the extra work performed by the respiratory muscles implies a more powerful stimulus to the respiratory centre. I have already shown(4, 6) that  $CO_2$ -tension changes in injected gases under the skin and in the abdominal cavity were con-

40 p.c. as when breathing  $O_2$  at 90.77 p.c. Since the  $O_2$  in physical solution depends only upon the  $O_2$ -tension in the air breathed there must have been nearly 2.3 times more  $O_2$  in solution in the blood when breathing  $O_2$  at 90.77 p.c. than with  $O_2$  at 40 p.c. If we assume that the alveolar  $O_2$ -tension was about  $\frac{1}{3}$ rd less than that in the inspired air, this will mean that the alveolar  $O_2$ -tensions were about 60 p.c. and 27 p.c. respectively, which figures indicate that the  $O_2$  in physical solution was about 1.11 volumes p.c. and 0.48 respectively. The experiment just described was repeated in another animal with similar results. My experiments thus prove that in the administration of  $O_2$  at high tensions either to patients or to athletes, the  $O_2$  in the blood and in the tissues will be increased even if the Hb is already fully saturated with  $O_2$ , the increase in the  $O_2$  in physical solution being sufficient as shown above to raise the tension in the tissues.

Leonard Hill and Flack(9) proved that breathing  $O_2$  at high tension was of benefit to healthy athletes during exertion. A. V. Hill, Long and Lupton(10) recently found that breathing  $O_2$  at 50 p.c. increased the  $O_2$ -intake during exertion. Both Leonard Hill and A. V. Hill and their co-workers considered that the heart was greatly relieved by breathing  $O_2$ . Leonard Hill and Flack believed that relief to respiration was also of great importance.

Recently(8), I found that under ordinary conditions following moderately severe muscular exercise, of about 10 minutes' duration in rabbits, the  $O_2$ -tension was increased by 10 mm., that is, from 25 to 50 p.c. throughout the tissues owing probably to the lactic acid formed exerting its effect upon the dissociation of  $HbO_2$ . This increase therefore resembled in its degree the effects produced in a resting animal by breathing  $O_2$  at about 40 p.c. for 3 or 4 hours, as described above. Muscular exercise is therefore an efficient method—and probably the natural method—to increase the  $O_2$ -tension in the tissues. In one respect the effects of muscular exercise differed definitely from the effects of breathing  $O_2$  at high tension. In the former the  $O_2$ -tension was as markedly increased under the skin as in the abdominal cavity whereas in the latter the  $O_2$ -tension was much less affected under the skin than in the abdominal cavity. It is obvious that there was a different factor or factors at work in the case of muscular exercise; probably lactic acid was the chief factor. Again the change produced by muscular exercise was more rapid, reaching its maximum in about an hour after the cessation of the exercise.

Returning to Table II to study the effects of breathing  $O_2$  at 90.77 p.c.

a definite rise in  $\text{CO}_2$ -tension in the tissues but produced no material effect upon the  $\text{O}_2$ -tension in the tissues nor upon the  $\text{O}_2$ -intake.

*Effects of  $\text{O}_2$  at high tension (above 20.93 p.c.).* I used  $\text{O}_2$ -mixtures varying from 38.30 to 90.77 p.c. Table II gives details of a typical experiment and Table III gives the average figures for seven observations in three animals.

From Table II it will be observed that breathing ordinary air through the apparatus for 37 minutes slightly increased the  $\text{O}_2$ -tension *i.e.* by 1 mm. under the skin and in the abdominal cavity, but breathing  $\text{O}_2$  at 90.77 p.c. produced the very marked rise of 26 mm. *i.e.* from 32 to 58, in the abdominal cavity but a much smaller rise under the skin. In other experiments the rise under the skin was quite definitely marked, being as much as 5 mm., but it was never so marked as in the abdominal cavity, probably because of the better circulation in the latter region. There was also a distinct lag in the changes under the skin, the  $\text{O}_2$ -tension still rising when that in the abdominal cavity was falling; it is possible that there was some mechanism at work in the case of the skin, opposing the rise of  $\text{O}_2$ -tension.

Other experiments proved that in the case of the abdominal cavity equilibrium of  $\text{O}_2$ -tension had become established in about 4 hours when breathing  $\text{O}_2$  at 90.77 p.c. and of course more rapidly with a lower  $\text{O}_2$ -tension in the inspired air.

On rebreathing of  $\text{O}_2$  at 20.93 p.c. the  $\text{O}_2$ -tension fell again in the abdominal cavity from 58 to 52 mm. in 2 hours (see Table II) and one day later it had returned to its normal figure of about 30 mm. whilst the  $\text{O}_2$ -tension under the skin was still above normal.

In Table III it will be seen that the average rise of  $\text{O}_2$ -tension in the abdominal cavity obtained in seven observations was from 35 to 52 mm. Hg. The separate experiments indicated clearly that the rate and degree of increase of  $\text{O}_2$ -tension varied directly as the extent of the increase in the  $\text{O}_2$ -tension in the air breathed. Thus in the experiment in Table II the  $\text{O}_2$ -tension in the abdominal cavity increased by 26 mm. in 4 hours when breathing  $\text{O}_2$  at 90.77 p.c. whereas in another experiment with the same animal and the same amount of gas in the abdominal cavity the breathing of  $\text{O}_2$  at 40 p.c. for 4 hours increased the  $\text{O}_2$ -tension by only 14 mm. This difference was due to the  $\text{O}_2$  in physical solution in the blood which of course must have been very much greater when breathing  $\text{O}_2$  at 90.77 p.c. than with  $\text{O}_2$  at 40 p.c.; it could not have been due to difference in saturation of the Hb with  $\text{O}_2$  because the Hb must have been as fully saturated when the animal was breathing  $\text{O}_2$  at

TABLE IV. Rabbit 2.8 kilo. About 300 c.c. gas in abdominal cavity and about 450 c.c. under the skin. Inspired air of decreased  $O_2$ -tension.

Time (mins.)	Tension under skin mm. Hg		Tension in abdominal cavity mm. Hg		$CO_2$ expired c.c. per min.	$O_2$ absorbed c.c. per min.	R.Q.
	$CO_2$	$O_2$	$CO_2$	$O_2$			
0 ( $O_2=20.93\%$ )	50	20	50	31	—	—	—
50	53	20	52	30	21.80	24.90	0.876
70 ( $O_2=11.00\%$ )	—	—	—	—	—	—	—
130	—	—	—	—	17.04	15.61	1.092
162	50	17	43	24	—	—	—
232	—	—	—	—	17.04	15.61	1.092
272	45	13	41	19	—	—	—
2 days later	50	23	53	30	—	—	—

TABLE V. Average figures for low  $O_2$  (11.33 %); five observations of at least 2 hours' duration.

Before	...	...	52	21	52	35	22.13	26.45	0.829
During	...	...	45	14	40	27	19.45	19.13	1.011

As might be expected, breathing  $O_2$  at 11 p.c. markedly reduced the  $O_2$ -tensions under the skin and in the abdominal cavity. On an average (see Table V) the  $O_2$ -tension was reduced from 21 to 14 mm. under the skin and from 35 to 27 mm. in the abdominal cavity. This was obviously due to decrease in saturation of Hb with  $O_2$  and also to decrease of  $O_2$  in physical solution in the blood.

Anoxæmia—Barcroft and others(15)—renders the respiratory centre more sensitive to  $CO_2$  so that one would expect the  $CO_2$ -tension to fall in the tissues as it does in the alveolar air; again  $CO_2$  would be more readily removed by the blood from the tissues as pointed out by Christiansen, Douglas and Haldane(11). It will be seen from Tables IV and V that this was the case in my experiments; on an average the  $CO_2$ -tension fell from 52 to 45 mm. under the skin and from 52 to 40 mm. in the abdominal cavity. Here again we have evidence that the changes in  $CO_2$ -tensions as examined by my method were similar to those obtained in alveolar air.

In Tables IV and V results for respiratory exchange are also given. As previous observers(13) found, breathing  $O_2$  below 12 p.c. reduced the  $O_2$ -intake; in my experiments it decreased by 28 p.e. on an average and by 37 p.e. in the observation illustrated in Table IV. The R.Q. was of course increased markedly owing to the removal of  $CO_2$  from the body, for the reasons stated above.

In Table VI I give details of an experiment in which the same animal was used under continuous observation to study the effect of breathing  $O_2$  at normal, high and low tensions, the results obtained being in com-

upon the  $\text{CO}_2$ -tension, we note that the higher  $\text{O}_2$ -content in the air breathed had a marked effect, producing an increase from 61 to 74 mm. under the skin and from 65 to 75 in the abdominal cavity. That this increase was real was proved by the fact that the  $\text{CO}_2$ -tensions decreased on shutting off the gas with high  $\text{O}_2$ -content and making the animal again breathe ordinary air through the apparatus; the  $\text{CO}_2$ -tension then fell from 74 to 67 mm. under the skin and from 75 to 62 in the abdominal cavity in two hours' time. This increase in  $\text{CO}_2$ -tension was observed in all the experiments with high  $\text{O}_2$  as will be seen from the average figures in Table III, the  $\text{CO}_2$ -tension increasing from 57 to 63 mm. under the skin and from 59 to 65 mm. in the abdominal cavity; these figures represent the condition of affairs after making allowance for the increase in  $\text{CO}_2$ -tension due merely to breathing through the apparatus. This rise of  $\text{CO}_2$ -tension in the tissues was probably due to the fact that blood takes up less  $\text{CO}_2$  if its  $\text{O}_2$ -tension is raised as shown by Christiansen, Douglas and Haldane(11); so that in my experiments the rate of removal of  $\text{CO}_2$  from the tissues was decreased during the breathing of  $\text{O}_2$  at high tension and therefore the  $\text{CO}_2$ -tension in the tissues was raised. It is possible that the respiratory centre was concerned in this rise of  $\text{CO}_2$ -tension although Haldane(12) has shown that the alveolar  $\text{CO}_2$ -tension is not materially affected when breathing high percentages of  $\text{O}_2$  during rest. Still there is some evidence that under certain conditions of strain  $\text{O}_2$  relieves the respiratory centre. Thus Leonard Hill and Flack(9) first proved in man that breathing  $\text{O}_2$  at high tension enabled a subject to tolerate a higher alveolar  $\text{CO}_2$ -tension when the breath was held, the highest figure they obtained being about 11 p.c., i.e. about 77 mm. Hg.

In Tables II and III will also be found the results for  $\text{CO}_2$ -output and  $\text{O}_2$ -intake whilst breathing gas with high  $\text{O}_2$ -content, i.e. up to 90.77 p.c. No definite effect was produced upon  $\text{O}_2$ -intake, confirming previous observers(13). The  $\text{CO}_2$ -output was decreased—confirming Leonard Hill and Macleod(14)—owing, no doubt, to the retention of  $\text{CO}_2$  in the tissues as explained above. The R.Q. fell slightly for the same reason.

*Effects of  $\text{O}_2$  at low tension (about 11 p.c.).* It has long been established that symptoms of anoxæmia do not occur until the  $\text{O}_2$ -tension in the air breathed has fallen to about 12 or 13 p.c. I therefore used gas mixtures with  $\text{O}_2$  at about 11 p.c., to study the changes produced in tissue  $\text{O}_2$ -tension. Table IV gives details of one experiment and Table V gives the average results of my five observations in four animals.

plete agreement with all that has been described above. The details of this experiment give some idea of the rate at which the changes were produced on altering the tension of  $O_2$  in the inspired air. The processes were slow even when the quantities of gas present in the animal were moderate; of course the quantity of gas present must influence the rate at which equilibrium was attained.

TABLE VI. Rabbit 2.8 kilo. About 200 c.c. gas in abdominal cavity and about 300 c.c. under the skin.

Time (mins.)	Tension under skin mm. Hg		Tension in abdominal cavity mm. Hg		$CO_2$ expired c.c. per min.	$O_2$ absorbed c.c. per min.	R.Q.
	$CO_2$	$O_2$	$CO_2$	$O_2$			
0 ( $O_2=20.93\%$ )	50	23	53	31	—	—	—
67 "	56	22	60	33	25.10	26.10	0.962
70 ( $O_2=40.95\%$ )	—	—	—	—	—	—	—
129 "	—	—	—	—	23.78	27.27	0.872
195 "	63	23	65	38	—	—	—
198 ( $O_2=20.93\%$ )	—	—	—	—	—	—	—
290 "	57	25	57	37	23.73	27.20	0.873
294 ( $O_2=11.40\%$ )	—	—	—	—	—	—	—
368 "	53	14	45	31	17.46	18.19	0.960
3 days later	49	28	49	36	—	—	—

In conclusion I refer briefly to the effects of change of barometric pressure upon the  $CO_2$ - and  $O_2$ -tension in tissue spaces. This was studied by Leonard Hill and myself (16) and where comparable the results were similar to those obtained in the present paper. The conditions of course were somewhat different since in the pressure experiments in the special chambers the whole body—including the injected gas—was submitted to the external physical changes as well as to the physical changes in the air breathed; in the present experiments only the composition of the air breathed was altered. The confirmation, however, indicates that the method of injection of gases has given reliable results.

#### SUMMARY.

(1) The effects of increasing the  $O_2$ -tension from 20.93 to 90.77 p.c. and of decreasing the  $O_2$ -tension from 20.93 to 11.00 p.c. in the air breathed, upon the  $O_2$ - and  $CO_2$ -tensions in gas injected under the skin and into the abdominal cavity have been studied.

(2) Increase of  $O_2$ -tension in the air breathed markedly increases, e.g. by 26 mm. the  $O_2$ -tension in the abdominal cavity owing to the increase of  $O_2$  in physical solution in the blood; a smaller rise is produced under the skin. Evidence is thus obtained that administration of  $O_2$ ,

even if the Hb is fully saturated, will increase the  $O_2$ -tension in the tissues. This probably explains the beneficial effect of  $O_2$  in healthy subjects during exertion. The  $O_2$ -tension in the tissues varies directly as that in the inspired air—within the limits tested.

(3) Decrease of  $O_2$ -tension in the air breathed markedly decreases the  $O_2$ -tension in the tissues.

(4) Increase of  $O_2$ -tension in the air breathed markedly increases the  $CO_2$ -tension in the tissues, probably because the increase of  $O_2$ -tension in the blood decreases the rate at which  $CO_2$  is removed from the tissues, perhaps the respiratory centre is also concerned.

(5) Decrease of  $O_2$ -tension in the air breathed markedly decreased the  $CO_2$ -tension in the tissues, the respiratory centre being rendered more sensitive to  $CO_2$ , and the  $CO_2$  being more readily removed from the tissues by the blood.

(6) Results of previous observers, for the effects of changes in  $O_2$ -tension in the air breathed upon the respiratory exchange, are confirmed, a modification, of the Douglas-Haldane method, in an unanæsthetised rabbit was employed.

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# THE RELATION IN DIURESIS BETWEEN VOLUME OF URINE AND CONCENTRATION OF A DIURETIC WITH THE INFLUENCE OF TEMPERATURE UPON IT.

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THE secretion of urine more concentrated than the blood, according to the rival theories of kidney secretion, is due either to the reduction of a large volume of glomerular filtrate to small bulk or to the secretion of a concentrated urine. The influence of temperature on the activity of the kidney, in addition to its intrinsic interest, holds out some prospect of discriminating between the two, as on the filtration absorption theory cooling (which we may suppose diminishes activity) should produce an increase in volume with a diminution of concentration whereas such an increase in volume would not necessarily occur if the urine were formed by secretion.

On consideration of the spontaneously changing rates of flow of urine, it was first thought desirable to find if any relation could be traced between the rate of flow and the concentration of a substance present in the blood in such amount as to form the predominant diuretic stimulus. Although the effect of cooling the animal does not give a clear decision on the problem set (largely owing to the possibility of vasomotor changes in the kidney) the experiments lead to results as to the relation between concentration and rate which is of interest and allows a value to be given to the influence of temperature on the activity of the kidney

During anæsthesia the kidney is under the influence of an excessive concentration of glucose in the blood. As previous experiments<sup>(1)</sup> gave indications of the excretion of glucose according to a definite rule attention was first directed to the relation between concentration of this substance and the volume of the urine at ordinary temperatures. The experiments were made on rabbits under urethane narcosis. The urine was collected from a bladder cannula and the necessary injections given through the jugular vein. To ensure an excess of glucose injections of 5 p.c. glucose were given in nearly all cases. The samples collected were of the order of 1 c.c. Both samples were taken from the carotid and the blood allowed to clot. The urine and serum were analysed for glucose



by the McClean method(2). The concentration of glucose in the serum was taken as equivalent to that in the plasma(1). A few observations were made with oxalated plasma, using formalin to prevent glycolysis(3).

Contrasting the flow of urine with the urine concentration it was found that the results with a range of urine flow of .05 c.c. per minute to 0.1 c.c. per minute could be expressed as  $\sqrt{V} \times C = K$  ( $V$  = c.c. of urine per minute and  $C$  the molecular concentration of glucose).  $K$  is practically constant for the individual rabbit. (Exp. 1, Table I.) Over the series of experiments on 46 rabbits  $K$  lies with very few exceptions between .10 and .18. This constant at first seemed independent of the plasma concentration. But considering that the blood glucose was generally small compared with the urinary glucose the formula might possibly be  $\sqrt{V}(C_u - C_B) = K$ . In order to decide between these alternative formulæ large injections of glucose were tried (Exps. 2, 3, 4). Table I gives results from these experiments.  $\sqrt{V}(C_u - C_B)$  remains a constant over very wide variations of rate of urine flow (.05 to over 3 c.c. per minute) and of concentration of glucose (.45 - 1.8 p.e.). Fig. 1 gives the results of plotting  $\sqrt{V}(C_u - C_B)$  against the volume of

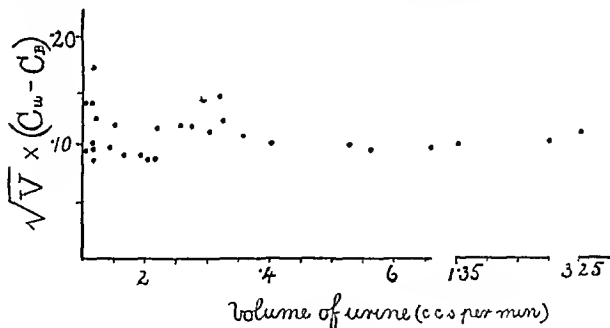


Fig. 1. See text.

urine. From the formula  $\sqrt{V}(C_u - C_B) = K$  we have  $C_u = \frac{K}{\sqrt{V}} + C_B$ . i.e., at a fixed rate of flow of urine there is but little increase in the glucose excretion with increase of blood sugar. Increased output of sugar on increasing the plasma concentration results mainly from the increased rate of flow.

The limits of applicability of the formula  $\sqrt{V}(C_u - C_B) = K$  have

to be considered. For slight degrees above the glucose threshold the formula does not necessarily apply. It applies beyond 0.43 p.c. glucose in the plasma (about 0.2 p.c. for the whole blood). The lowest limit of rate of flow of urine at which the formula applies is approximately .05 c.c. per minute per kidney. Below this rate the concentration does not in general increase, so that all rates below .05 c.c. may apparently be regarded as .05 c.c. in using the formula for comparison of the values of  $K$ . The constant for different animals varies from .09 to .17, i.e., a variation of about 30 p.c. from a mean figure 0.13. The establishment of these formulæ allows the activity of the kidney at different temperatures to be contrasted. An injection of about 30 c.c. of 5 p.c. glucose was given, and the animal cooled by exposure, a cooling of 10 degrees being produced in about an hour and a half. Samples of urine were taken during the cooling and the temperature in the peritoneal cavity noted. The blood pressure was recorded. In Fig. 2  $K$  of the formula  $\sqrt{V} \times C$

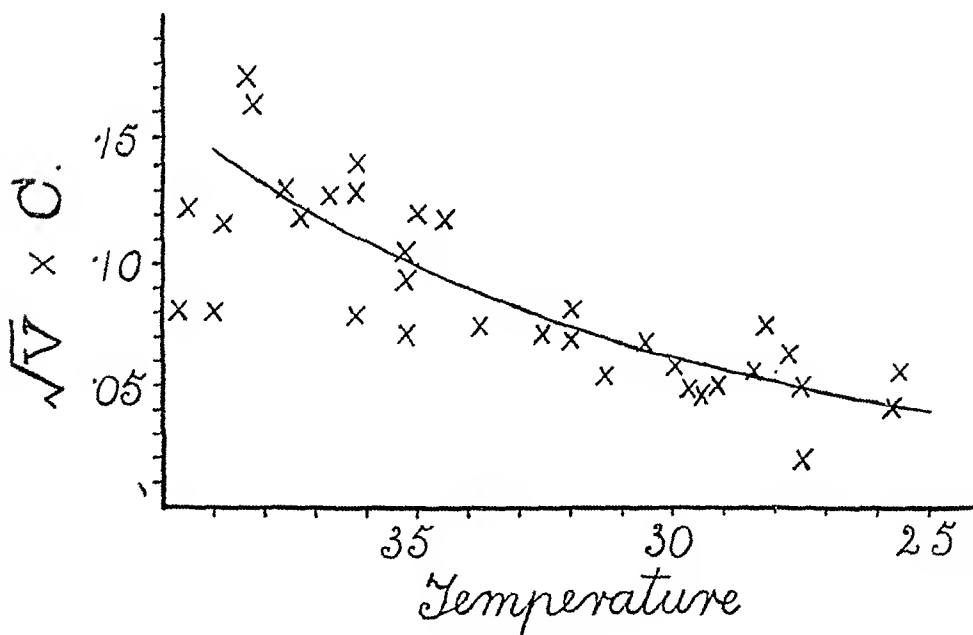


Fig. 2. See text.

for all experiments is plotted against the temperature. It will be seen that the value of  $K$  at 38 is about 2.6 times as great as at 28, or 10 p.c. per degree increase in the concentrative power for glucose. Unfortunately blood estimations were not made in a sufficient number of cases to plot results with the more accurate second formula. A few experiments (as

in Exp 5, Table I) where this formula is used confirm the relation established between the activity constant and the temperature. As

TABLE I

Exp	Injection	Temp	vol of urine in c c per min	% glucose in urine	% glucose in serum	$\sqrt{V} \times C$	
1	37 c c 5 %	38.5	516	4.0		16	
			344	4.9		16	
			247	6.5		18	
			227	6.8		18	
			188	7.45		18	
			186	7.5		18	
			123	9.3		18	
			100	9.5		17	
						$\sqrt{V} \times (C_u - C_s)$	
2	25 c c 5 %	37.3	527	3.32	77	103	
			402	3.72	77	104	
	80 c c 5 %		1504	3.36	1.8	107	
			1353	3.38	1.8	102	
3	30 c c 5 %	36.5	562	2.95	57	099	
	80 c c 5 %		325	2.75	1.55	120	
4	20 c c 5 %	37	321	4.2	315	122	
			66	3.34	1.16	099	
5	50 c c 5 %	36.8	046	11.1	51	126	
	$\frac{1}{2}$ hr before col- lection of urine)	32.8	111	5.2	60	085	
		30.3	04	6.1	47	070	
		28.1	011	6.6	59	075	
		25.2	023	4.9	59 (?)	053	

already stated, if the blood sugar be near the threshold, *i.e.*, below 0.43 p c in the plasma, the formula is no longer applicable. The concentration of glucose in the urine will not then be an index of the concentrative power of the kidney for glucose. On cooling such an animal the glucose in the urine may rise following an increase of the blood sugar. For example

Rabbit	wt. 1.7		
Temp	Glucose in urine	Rate of urine	Glucose in serum
37.35	0.31 p c	0.04	40 p c
30.35	4.68 p c	0.16	46 p c

*Experiments with chloride* It is obviously desirable to confirm these results with other urinary constituents, and chlorides present additional points of interest. A series of experiments were consequently done in which by injections of about 50 c c 2 p c NaCl it was ensured that the prominent stimulus to the kidney activity was the excretion of chloride. At the beginning and towards the end of the diuresis samples of blood (about 5 c c) were usually taken into tubes containing some potassium

oxalate powder. They were corked at once, the tube being entirely filled with blood apart from a small bubble of air for mixing. The blood was then centrifuged and the plasma analysed for chloride by the method described by Whitehorn(4), 1 c.c. of plasma was generally used for the analysis. The urine was analysed for chloride by the Volhard method. Fig. 3 gives the relations of rate of flow and concentrations of

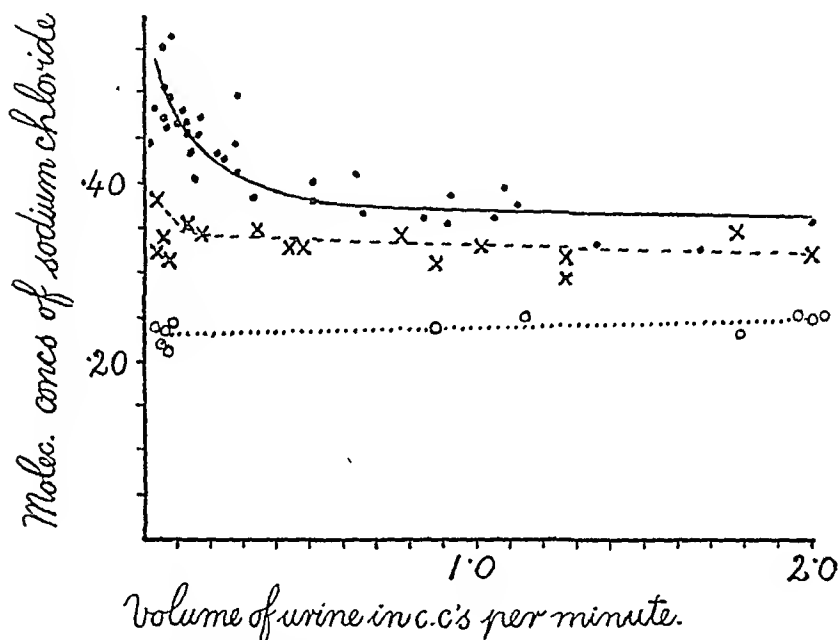


Fig. 3. Dots—concentration of NaCl in urine at body temp. of about 37° C.  
 Crosses—concentration of NaCl in urine at body temp. of about 27° C.  
 Dotted circles—conc. of NaCl in plasma.  
 All concentrations expressed as NaCl  $\times$  2.

chloride in urine with those of the blood plasma. It will be seen that the concentration of chloride rises as the rate falls.

If we direct attention for the moment to the maximal chloride concentration attained, we find that this is not altogether dependent on the rate or on the concentration of chloride in the plasma. A maximum concentration might be expected when the flow is low and when the concentration of chloride in plasma is well above the threshold, but if we take all observations taken under all conditions in which the rate of flow is below 0.1 c.c. per minute and plot the urinary concentration against the plasma concentration (Fig. 4) we find that the concentration of urine may be a maximal value with very low plasma concentrations. When,

however, we limit ourselves to experiments in which an ample injection of chloride has been administered we find there is a distinct relation

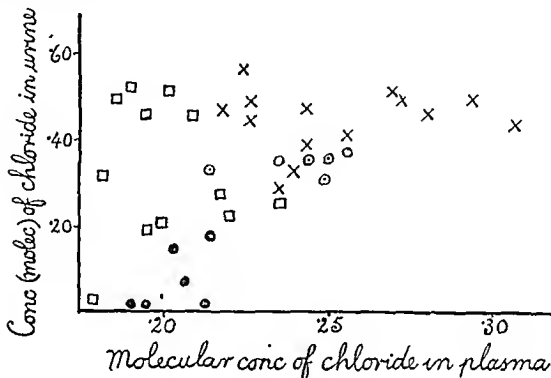


Fig. 4. See text.

Squares—NaCl concentration in urine at body temp. 37° C. no injection.

Crosses—NaCl concentration in urine at body temp. 37° C. NaCl injection.

Black circles—NaCl concentration in urine at body temp. 27° C. no injection.

Dotted circles—NaCl concentration in urine at body temp. 27° C. NaCl injection.

between the rate of flow and the urinary concentration. On Fig. 3 these are plotted with comparable injections (about 50 c.c. 2 p.c. NaCl) as in these the blood chloride concentrations are similar. On analogy with glucose one would expect that the expression  $\sqrt{V}(C_u - C_B) = K$  would apply. It is found, however, that  $\sqrt{V}(C_u - 1.25C_B) = K$  is more in agreement with the results. A curve constructed from this formula is seen in Fig. 3 where  $\sqrt{V}(C_u - 1.25C_B) = .057$  ( $V$  being c.c. per minute per kidney and concentrations being expressed as molecular concentrations multiplied by two). If this same formula be used for still larger injections (150 c.c.) in which the plasma chloride is considerably increased, it is found to be in satisfactory agreement with the results.

It is suggested that the regularity of the behaviour of the chloride in these cases depends upon the fact that the stimulus to the kidney is the excretion of chloride and that in animals which have received smaller injections of chloride, or to which no injection was given the volume of the urine is related to and governed by concentrations of some other constituent—possibly glucose or urea.

We are now in a position to consider the influence of temperature on the excretion of chloride (70 c.c. of 2 p.c. saline), at a low temperature. As in the experiments with glucose we should expect a reduction of  $K$  in the formula  $\sqrt{V}(C_u - 1.25C_B) = K$  to  $\frac{K}{2.6}$  for 10 degrees. Constructing a curve on such a hypothesis it will be found to agree with the experimental results as shown in Fig. 3. Even in the extreme case of a rate of 4.3 c.c. per minute per kidney with a blood chloride of .74 and a temperature of 28.9° C. the predictable concentration is .96, the actual concentration .95. It is interesting to note that with such an extreme diuresis and depressed concentrative power it might be expected that the urinary chloride should be close to that of the blood. It is likely, however, that a membrane effect of the Donnan type is taking place. This might also account for the factor 1.25 in the formula

$$\sqrt{V}(C_u - 1.25C_B) = K.$$

The effect of cooling without any chloride injections is to cause in general a great fall or total disappearance of chloride from the urine. This is altogether out of proportion to the effect upon the maximal concentration or on the constant  $K$ . Table II illustrates the results on the chloride excretion of cooling (13 exps.) in which no injections of chloride were given. We see that in general the chloride almost disappears from the urine. This is associated with a slight diminution in the blood chloride. This slight diminution does not account for the marked effect on the urinary concentration, as the blood plasma may still be well above the threshold. The result may be explained by a relatively increased

TABLE II.

Exp.	Vol. of urine in c.c. per min. per kidney	% NaCl in urine	% NaCl in plasma	Temp.
1	.015	.56	.56	38.15
	.045	.09	.57	30.3
2	.023	1.3	.60	37.9
	.043	0.2	.59	29.4
3	.08	.70	.65	37.9
	.04	.02	.61	28.1
4	.05	.46		38.8
	.008	.12		29.1
5	.08	.07		37.1
	.06	.045		32.2
6	.044	.18		38.3
	.035	.42		31

secretion of some other urinary constituent at the low temperature which with its water of secretion depresses the concentration of chloride

Experiments in which at ordinary temperatures the urinary chloride was below the concentration of the blood present a possibility of recognising a combined filtration and absorption process. If filtration and absorption are occurring in such cases the chloride concentrations of the urine should rise on cooling. The results generally show in such cases also a pronounced fall in the chloride from which one may conclude a filtration absorption process was at least not predominant. Three experiments like Exp 6 of Table II however show a rise in chloride concentration on cooling.

*Experiments on urea* Some experiments were performed with urea to see if it behaved similarly to the chloride and the glucose. Three experiments were performed in which 60 c c of 5 p c urea were injected into a rabbit at ordinary temperatures and the urine collected as before and analysed by the hypobromite method. Two experiments were performed where the animal was cooled to a low temperature and 60 c c of 5 p c urea were injected as before. Table III gives the highest concentrations of urea reached in each experiment with the corresponding rates of flow and temperature. Assuming that the formula  $\sqrt{V} \times C$  applies for a small range of rate in these cases for urea as well as it does for sugar in a glucose diuresis the influence of temperature on the excretion of urea may be estimated at about 10 p c rise per degree in concentrative power.

TABLE III

Exp	Vol of urine in c c per min per kidney	% urea in urine	Temp	$\sqrt{V} \times C$
1	034	1.375	24.0	0459
2	057	1.75	28.0	0696
3	084	3.6	38.1	173
4	074	3.15	37.8	143
5	073	3.15	38.2	140

*Influence of cooling on the rate of flow of urine* The effect of cooling, other things being equal, should increase the rate of flow if filtration with absorption prevailed or might diminish it if the rate of urine be formed by a secretion process. It was found that the urine rate was increased by cooling but that there was a corresponding increase in the blood-pressure in those experiments in which it was observed. It is true that the increase in flow was larger than the increase in blood-pressure, but although it has been shown that the rate of urine follows in many cases the blood-pressure there is no evidence that it is directly pro-

portional to it, and indeed owing to the fact that the uriniferous tubules are not rigid but elastic a small change in pressure might account for a disproportionately large change in rate(5). If the cooling continues the blood-pressure falls and when it has reached its original level there was in three out of four experiments a decided decrease in rate of flow. In one experiment there was a small increase.

TABLE IV.

Exp.	Temp.	Blood-pressure mm. Hg.	Rate of urine in c.c. per min. per kidney
1	36.5	68	.056
	33.0	85	.070
	28.5	68	.012
2	37	92	.050
	35	100	.108
3	36.5	105	.084
	35	120	.114
	30	105	.048
4	36.5	105	.040
	33.0	136	.082
	30.5	105	.030
5	36.5	100	.07
	34.5	158	.10
	24.5	100	.082

### Discussion.

It will be seen that the equations  $\sqrt{V}(C_u - C_B) = K$  for the excretion of glucose and  $\sqrt{V}(C_u - 1.25C_B) = K$  for chloride used in the consideration of the temperature effects, resemble the formula Austin, Stillman and Van Slyke have proposed for urea  $\sqrt{V}C_u = KC_B$  in that the value  $K$  depends on the square root of the volume. It was found in these experiments that the formulæ Ambard proposed for the excretion of urea were totally inapplicable. The formulæ proposed here relate only to cases in which the concentrations considered are those of the substances provoking the diuresis. In a previous communication(1) the effect on the glucose excretion of the injection of other substances was investigated, and it was shown that except with injections of normal saline the glucose excretion was depressed. In those cases of chloride output in which the formula does not apply the blood chloride is not much above the threshold and a direct interference by some other urinary constituent may be responsible for the divergence; or it may be that such a constituent is leading the diuresis according to the formula, though not directly interfering with the chloride excretion. In the latter case the



chloride would appear in a greater volume of urine and its concentration be depressed.

It has been noted by Barcroft and Straub<sup>(7)</sup> that with saline diuresis there is no increase in the  $O_2$  consumption by the kidney. This they have used as an argument in favour of filtration. It is possible, however, that a constancy of available energy is responsible for the reduction in concentration of chloride with increased rate, and that some such limitation of available energy underlies the empirical formula advanced. It is probable that during such a diuresis the main work of the kidney consists in excreting the diuretic substance, and it is in any case interesting to find that the reversible work done in unit time on glucose in a glucose diuresis as calculated from the experimental values varies but little with the rate. Thus the average calculated value in these experiments for the work done by the kidney in excreting glucose at a rate of 0.1 c.c. urine per minute is .0176 kilogram-metre, at 0.5 c.c. per minute .0155, and at 1.4 c.c. per minute it is .0120.

The effect of temperature on the *maximal* concentration is not the same for chloride as for glucose and urea. The maximal concentration for chloride is only 3 p.c. higher per degree whereas with urea and glucose the increase is approximately 10 p.c. per degree. If from the maximal concentration we subtract the plasma concentration; or with chloride the plasma figure multiplied by 1.25 as in the formula, the distinction disappears, and the temperature effect for the three substances is the same, being approximately 10 p.c. per degree rise in concentration over the range of temperature examined.

I was not aware until my attention was directed by the Editor to a reference inaccessible to me that experiments with a similar object had previously been done by Hallion and Ambard (*Physiol. et Path. des Reins*. 2 edit., p. 77. 1920), who found, it appears, that in the dog a decrease in concentration of urea on cooling and a not inconsiderable increase in rate of secretion without however mentioning the blood-pressure.

#### SUMMARY.

1. In an animal which has received a large injection of glucose it is found that the rate of flow of urine and the concentration of glucose are related to one another by a formula  $\sqrt{V} (C_u - C_B) = K$  ( $V$  = volume of urine,  $C_u$  and  $C_B$  being concentrations in urine and in blood plasma).

2. A similar formula  $\sqrt{V} (C_u - 1.25C_B) = K$  is found to apply to the concentration of chloride and the rate of flow of urine in a saline diuresis.

3. The effect of body temperature on the constants in these formulæ is to cause an increase of 10 p.c. per degree. A similar increase is found with urea.

4. The effect of the temperature on the *maximal concentrations* of glucose and urea is an increase of approximately 10 p.c. per degree. With sodium chloride the effect is 3 p.c. per degree.

5. In animals which have received no injection and in which the salt concentration at normal temperature is below the blood concentration, lowering the temperature causes generally a falling salt concentration and not an increase as might be expected from the absorption theory of the formation of urine.

6. With similar blood-pressures the rate of urine is not greater at a low body temperature (about 29° C.) than at a high (about 38° C.).

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THE DIRECT ACTION OF INSULIN ON FAT  
METABOLISM. BY H. S. RAPER AND E. C. SMITH<sup>1</sup>,  
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THE possibility that insulin might, in addition to its influence on carbohydrate metabolism, exert a direct action on fat metabolism, is suggested by the fact of the immediate reduction of lipæmia and acetonuria in the diabetic on administration of insulin (Fonseca<sup>(1)</sup>, Killian<sup>(15)</sup>).

Direct evidence of the actual synthesis of fat under the action of insulin has never been obtained. Dudley and Marrian<sup>(2)</sup>, comparing the fatty acid content of livers of normal mice and of mice brought into convulsions by insulin, concluded that no synthesis of fat had taken place in the liver. Kellaway and Hughes<sup>(3)</sup>, by the indirect evidence of a rise in the respiratory quotient after insulin in a normal person, which could not be accounted for by increased combustion of carbohydrate, came to the conclusion that a complex poorer in oxygen must have been formed, pointing to a probable synthesis of fat. On the other hand, Buru and Dale<sup>(4)</sup>, using the decapitated, eviscerated cat, could find no evidence from the respiratory quotient that the disappearance of sugar under the action of insulin is associated with a synthesis of fat.

An indication that insulin may have an effect on fat metabolism is afforded by the results obtained by Allan, Bowie, Macleod and Robinson<sup>(5)</sup>, working with totally depancreatized dogs kept alive with insulin. A preliminary fall in the fat content of the liver was followed by a large increase, in one case resulting in a fat content of 37 p.c. of the moist weight of the liver.

In view of these apparently conflicting results it seemed necessary to try and establish by a more direct method whether insulin caused any change in the total fat content of the whole animal which could be associated with the disappearance of sugar from the blood. Mice appeared to be suitable animals for this purpose because of their small size which permits of an estimation of the total fat in the body. Especially since Terroine and Weill<sup>(6)</sup> have found that after 36 hours' fasting, the fat content of mice is brought down to a figure which deviates but little

<sup>1</sup> Working for the Medical Research Council.

from the mean value from a series of animals so treated. This was not our experience. The amount of fat present in the starved animals and those starved and treated with insulin was so variable that it gave no hope that direct evidence of fat changes would be got in this way.

Six mice from the same litter were taken, starved 36 hours and weighed. Three of them were killed with chloroform, and the fatty acid content in the entire body estimated by the Liebermann method (Leathes<sup>(13)</sup>). The remainder were injected, each with 0.5 mg. insulin (prepared in the laboratory) in 0.17 c.c. water, subcutaneously. Each was killed in convulsions three hours after injection and the fatty acid determined. In the three controls the percentage of fatty acid was 5.84, 4.48 and 2.02. In those injected with insulin it was 6.22, 3.02 and 2.64.

In default of a method capable of indicating an absolute increase in total body fat, experiments were devised in which the variations could be followed in liver and skeletal muscle, the tissues in which a change, if any, would most be expected. These were found to be most conveniently carried out on the decerebrate cat, and the greater part of the experiments here recorded were carried out on that preparation.

Previous work on the effects of insulin on decerebrate cats has been carried out by Olmsted and Logan<sup>(7)</sup>, who found that the effects of insulin were dependent on the removal of the pituitary body, confirming Burn<sup>(8)</sup> that pituitrin inhibits the hypoglycæmic action of insulin. The pituitary body was therefore dissected out in every case immediately after decerebration. Nevertheless in only 20 p.c. of the animals was a hypoglycæmic reaction elicited. In many of the decerebrate animals convulsions and vomiting occurred, but these were in only a few instances more definitely marked than in the controls when no insulin was given, and were mainly due to difficulty in breathing. They were not associated with hypoglycæmia. Typical insulin convulsions which could be definitely correlated with the lowering of blood sugar below 0.05 p.c. occurred only in 10 p.c. of the animals. The convulsions usually passed off with the rise in blood sugar which they apparently occasioned. The cat is known to be rather more resistant to insulin than the rabbit (Houssay, Sordelli and Mazzocco<sup>(9)</sup>), hence proportionately larger amounts were expected to be necessary to produce the required fall in blood sugar, but after gradually increasing the dose from 3 to 15 clinical units per kilo. body weight, it was found that the smaller doses were as effective as the larger, the latter merely causing the rapid death of the animal with only a very slight fall in blood sugar, one case only excepted. The effectiveness of the dose is completely dependent on the initial glycogen content of

the liver This is shown by the results collated from the several experiments in Table I

TABLE I The behaviour of the blood sugar consequent on the action of insulin is indicated thus +, a slight fall, ++, a marked fall, H, hypoglycæmia, i.e. sugar below 0.1 p.c., -, a slight rise, --, a marked rise, n.c., no change

Exp	Blood sugar	Glycogen	Exp	Blood sugar	Glycogen
1	-	5.0	13	+	0.5
2	n.c.	2.4	14	n.c.	0.4
3	--	2.0	15	++H	0.3
4	n.c.	2.0	16	-	0.3
5	n.c.	2.0	17	++-H	0.2
6	+--	1.5	18	+	0.2
7	-	1.3	19	+	0.2
*8	++-H	0.9	20	++H	0.2
9	+	0.9	21	++H	0.1
10	+	0.8	22	++H	0.1
11	n.c.	0.75	23	++H	Trace
12	n.c.	0.7	24	++H	Trace

\* Received 10 units insulin per kilo

It will be seen that with very high glycogen content the effect of insulin from the commencement was negative, or, in two instances, seemed to be the cause of a considerable rise in blood sugar. This does not appear to coincide with McCormick's results (10) on the rabbit, in which the fall of blood sugar is at first independent of the glycogen content of the liver and the dose of insulin, but the behaviour after the first hour is then governed by these. The failure to obtain a fall at the outset in the decerebrate cat may be due to the antagonistic effect of the ether and operation, which occasion a very high initial blood sugar percentage (Mellanby (11)) usually lying between the limits of 0.26 and 0.40 p.c.

Since the effectiveness of the insulin was found to depend so completely on the glycogen content of the liver, attempts were made to ensure that the glycogen should be reduced as much as possible, by preliminary starvation and exercise, and by feeding on an almost exclusively protein diet (codfish) for a week in order to induce fat rather than glycogen storage, but in only a limited number of animals was the liver thus rendered almost glycogen free. In every one of these cases, even with small doses of insulin, the blood sugar was reduced from an initial value averaging 0.3 p.c. to 0.1 p.c. or below, in the remainder only a slight fall, no change, or a rise in blood sugar occurred, depending mainly on the glycogen content of the liver and only to a very slight extent on the insulin dosage. The same difficulties in eliciting a response to insulin were encountered by Kleitmann and Magnus (12) in their preparations.

*Comparison of the amount of fat in different parts of the liver and in corresponding muscles of the hind limbs.*

The animals, starved for 24 hours, were anæsthetised with ether, decerebrated, and the pituitary body destroyed<sup>1</sup>. Comparison was made of the fatty acid in the left lateral lobe of the liver (which was found to be easily removed after ligation without subsequent hæmorrhage) and either the right central lobe or the whole remaining part of the liver. The muscles compared for fatty acid content were the semimembranosus and adductor femoris, cleaned on the two sides from all visible connective tissue fat. The left lobe and right hind limb were taken immediately after decerebration and the right lobe and left hind limb after 4 hours, or earlier if death occurred. The fatty acids were estimated by the Liebermann method, and to allow for possible changes of the tissues between the taking of the initial and final samples, the total nitrogen was estimated and the amounts of fatty acid calculated on the assumption that the nitrogen content had not changed. The weight of fatty acid was checked by titration with *N*/10 alkali in 50 p.c. alcoholic solution.

TABLE II. Left lateral and right central lobes of the liver.

Exp.	Fatty acid p.c.		p.c. difference. Right from left
	Left	Right	
25.	8.37	8.82	5
26.	4.06	4.20	3
27.	16.73	15.40	-7
28.	3.93	4.08	4

TABLE III. Left lateral lobe and the whole of the remaining liver.

Exp.	Fatty acid p.c.		p.c. difference. Rest from left
	Left	Rest of liver	
29.	6.10	6.20	2
30.	5.07	4.97	- 2
31.	5.19	4.68	- 10
32.	5.03	4.69	- 9
33.	7.25	7.07	- 3

TABLE IV. Right and left muscles of hind limb.

Exp.	Fatty acid p.c.		p.c. difference. Left from right
	Right	Left	
34.	1.04	1.01	- 3
35.	1.32	1.29	- 3
25.	2.35	2.40	2
36.	2.10	2.26	8
37.	1.56	1.56	0
38.	2.64	2.74	4
39.	1.37	1.37	0
33.	1.92	1.96	2

<sup>1</sup> We are indebted to Dr B. A. McSwiney for the decerebrate preparations.

These results show that the fatty acid content of the left lateral lobe of the liver and of the muscles on one side may be taken as showing within narrow limits the fatty acid content of the rest of the liver and of the corresponding muscles respectively. It will be noticed that our results differ from those of Dowler and Mottram(14), who found in some cases considerable differences in the fatty acid content of different parts of the liver of animals to which large quantities of fat had been administered.

*The effect of insulin on liver and muscle fat.*

The experiments showed that whether insulin had any certain effect or not depended on whether hypoglycæmia was or was not produced. Hypoglycæmia was taken as produced when the blood sugar fell below 0.1 p.c. Table V shows the result on the liver when there was no hypoglycæmia, and Table VI when it occurred. The amount of insulin injected is given in clinical units per kilo. body weight. Burroughs-Wellcome insulin was used in these experiments.

TABLE V. Liver, no hypoglycæmia

Exp	Units	Blood sugar	Fatty acid p.c.		p.c. diff.
			Left	Right	
13	30	+	20.72	23.48	20
10.	30	+	15.76	17.10	8
3.	45	- -	3.08	3.10	0
6.	40	+ - -	11.79	12.06	2
14.	45	n.c.	15.33	11.60	-20
4	60	n.c.	3.15	3.22	+
2.	100	n.c.	9.30	8.40	-10

TABLE VI. Liver. Hypoglycæmia.

23.	45	+ + -	16.78	14.79	-12
17.	45	+ + -	2.76	2.16	-22
24.	50	+ + -	3.79	3.42	-10
8.	100	+ + -	6.13	5.45	-11
12.	45	+ +	24.90	21.52	-17.5
22.	45	+ +	8.56	7.80	-9
15.	45	+ +	31.80	29.58	-7
20.	45	+ +	6.17	5.59	-10

TABLE VII Muscle. Hypoglycæmia

40.	30	+ + -	1.62	2.03	24
24.	50	+ + -	1.21	1.36	12
21.	45	+ +	1.18	1.24	5
22.	45	+ +	1.33	1.43	7.5
15.	45	+ +	1.56	1.76	13
20	45	+ +	0.90	0.83	-8

Insulin which did not produce hypoglycæmia (six experiments) had also no definite effect on the fatty acids in muscle. The percentage variation was from  $\pm 8$ , i.e. not greater than in the controls. On the

other hand, when hypoglycæmia was produced there was in all cases but one a distinct increase in the muscle fat. The results are given in Table VII.

In order to determine whether any concurrent changes were taking place in the amount of fat in the blood, estimations of this were carried out in a few instances. The first sample was taken from the carotid artery, the second from the heart, this being necessitated by the low blood-pressure of the insulin animal three hours after decerebration, the withdrawal of 3 c.c. from the carotid artery being a matter of considerable difficulty.

In the control animals the blood fat was 0.02 and 0.11 p.c. less in the second than in the first sample. In two animals which received 4.5 units of insulin per kilo. and in which there was no hypoglycæmia the blood fat was 0.12 and 0.14 less, a decrease which cannot with certainty be attributed to the insulin. But in three experiments in which the same amount of insulin was given, but in which there was marked hypoglycæmia, the p.c. fat in the second sample was in each case greater than in the first, viz. 0.17, 0.09 and 0.09 p.c. It appears then that if insulin lowers the blood sugar below the normal level, it increases slightly the fat in the blood.

The evidence derived from the above experiments definitely disposes of the question of synthesis of fat in the liver associated with the fall in blood sugar occasioned by insulin. That the same is true of muscle is suggested by the absence of any definite change in the fat content of muscle during a fall in blood sugar which does not reach the stage of hypoglycæmia. This view has been considerably strengthened by carrying out a series of experiments following the method of Burn and Dale<sup>1</sup>. By using the decapitated and eviscerated preparation a large disappearance of glucose can be caused and its amount approximately estimated. Consequently the conditions are such that if fat were formed from this sugar it should be easily detected.

The animals used were not previously treated in any way. The decapitation was performed under ether by Sherrington's method, and the evisceration was carried out as described by Burn and Dale, the whole of the alimentary canal, between ligatures on the œsophagus immediately below the diaphragm and on the rectum, being removed. The ligaturing of the cœliac axis artery and the portal vein resulted in the exclusion of the liver from the circulation. The kidneys were also tied off and removed.

<sup>1</sup> We are much indebted to Dr Dale for making this suggestion.



During the experiment 5 p.c. glucose in normal saline solution was infused into the external jugular vein. After a preliminary 1-hour period of infusion at the rate of 2 c.c. per 10 minutes, 10 units (0.5 c.c.) insulin were injected into the right internal saphena vein. The right hind limb was then amputated and the fat estimated in a sample of muscle as in the first series of experiments. The rate of infusion was raised to 6 c.c. per 10 minutes. The blood sugar, however, fell in every instance as a result of the insulin injection. The amount of glucose disappearing during the ensuing period in excess of that calculated from the rate of disappearance in the initial period, lay, in six experiments, between 1 gm. and 3.5 grms. Assuming complete transformation into fatty acid, this would result in an increase in muscle fat of 0.1 to 0.3 p.c. or 8 to 20 p.c. of the fat content. Such an increase would be easily distinguishable by the method used. The following results were obtained.

TABLE VIII. Muscle.

Exp.	Excess glucose gm.	Fatty acid p.c.		p.c. diff.
		Right	Left	
41.	1.0	1.43	1.57	10
42.	3.0	1.44	1.44	0
43.	2.0	1.15	1.15	0
44.	3.5	1.78	1.62	-10
45.	2.2	1.95	2.00	2.5
46.	3.0	1.06	1.12	5

These figures provide no evidence for the formation of fat in muscle under the action of insulin.

### Discussion.

The evidence definitely disposes of any synthesis of fat in the liver associated with the fall in blood sugar occasioned by insulin, but it does not account for the diminution in the amount of fat in this organ which is associated with the extreme effects of insulin. The fact that under these conditions the fat content of the blood increases suggests a discharge of fat from the liver, but it is not legitimate to relate this quantitatively to the fat increase in the blood when it is not known what is happening to other fat depots. Brugsch, Horsters and Shiuoda(18), however, consider that a certain amount of the glucose formed in the liver in insulin hypoglycæmia must, on account of the low R.Q. of that organ, be derived from fat. The effects of insulin on the blood fat appear to depend on its effect on the blood sugar. If the sugar is decreased from a hypernormal value to normal, but not below, then the fat concentration also diminishes. But if the blood sugar falls below the normal,

then the fat content increases. This is the usual condition in the blood associated with a fatty infiltration of the liver, but in the experiments described in this paper this does not occur, on the contrary the reverse happens. It is, therefore, not possible from what we know at present, to correlate the changes occurring in blood fat with those occurring in the liver. It is of interest to note, however, that pituitrin, which antagonises insulin (Burn(8)), causes a fatty infiltration of the liver (Coope and Chamberlain(17)), whereas insulin may produce the reverse change.

That no synthesis of fat occurs in muscle during a fall in blood sugar which does not reach the stage of hypoglycæmia is evident from the results obtained with the decapitate eviscerate animals. Hence the large amounts of glucose disappearing under these conditions are definitely not converted into fat. But the meaning of the increase in fat content of muscle which occurs when the effects of the insulin are extreme, is not clear. It is accompanied by an increase of fat in the blood. Although muscle may be looked on as a fat depot (Terroine(16)) and may be storing fat under these conditions, it is not easy to see why the fat in the liver should not increase at the same time. The fat which is stored in muscle by well-fed animals is in all probability laid down, not in the muscle fibres, but in the connective tissue between the fibres. It is possible also that the fat cells in this tissue have the ability to synthesise fat from carbohydrate. That they may do so in the acute conditions of carbohydrate metabolism produced by insulin is possible, but it would be unwise to give any weight to this suggestion until it has been proved experimentally that adipose tissue cells can carry out this synthesis.

#### SUMMARY.

1. Insulin causes hypoglycæmia in decerebrate cats from which the pituitary body has been removed, only in animals with poor glycogen reserve.

2. In decerebrate animals with insulin hypoglycæmia fat disappears from the liver to the extent of 10 p.c. of the initial fat content and concurrently increases in muscle to the extent of 10 p.c. It also increases in the blood. These effects do not occur until the blood sugar concentration falls below 0.1 p.c. They appear to be associated only with the extreme effects produced by insulin.

3. In decapitate eviscerate animals with constant infusion of glucose no change in the fat content of muscle occurs under the action of insulin.

4. The evidence obtained does not support the view that fat synthesis in the liver is associated with insulin hypoglycæmia (confirming Dudley and Marrian(2)).

We desire to express our thanks to the Government Grant Committee of the Royal Society for a grant which has defrayed a part of the expenses of this investigation.

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# ON HÆMOCHROMOGEN AND THE RELATION OF PROTEIN TO THE PROPERTIES OF THE HÆMOGLOBIN MOLECULE.

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## PART I. HÆMOCHROMOGEN.

HÆMOGLOBIN is a conjugated protein consisting of globin and a non-protein part, containing pyrrol nuclei and iron, which we shall call hæm. (Its hydrochloride is known as hæmin.) Hæmoglobin can exist as such near the neutral point only. Dilute acid or alkali changes it into hæmochromogen<sup>2</sup>. This change is usually interpreted as being a separation of hæm from globin, and hæmochromogen is considered to be the reduced form of hæm. We intend to show that this separation does not occur and that hæmochromogen itself (and hence its oxide hæmatin) is a conjugated protein—a globin compound.

I. *The nature of hæmochromogen.* The first hint one has that hæmochromogen is a protein is that, in contrast to hæm, it is precipitated by protein precipitants. Further, hæmochromogen, like all proteins, is much less soluble around the neutral point than in acid or alkali, while hæm is soluble in alkali only.

Conclusive evidence that hæmochromogen is not simply reduced hæm is that it can be synthesised from reduced hæm by adding a protein to it. When a few crystals of hæmin are dissolved in dilute NaOH and a little Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> added as a reducer, reduced hæm is formed—a substance whose absorption spectrum, colour, and solubilities are entirely different from those of hæmochromogen. If now globin<sup>3</sup> is added the substance formed is indistinguishable from the hæmochromogen prepared by the action of caustic soda on reduced hæmoglobin. Other proteins, amino-

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<sup>2</sup> In the presence of oxygen, if no reducing agent is present, of course, hæmochromogen is oxidised to hæmatin and therefore general considerations about the nature of hæmochromogen will apply to hæmatin.

<sup>3</sup> Bertin-Sans and de Moitessier (5) were probably the first to show that the presence of protein is necessary (but cf. Hoppe-Seyler in *H.-S. Ztsch.* 2. 154). However, they do not appear to have noticed that combination between reduced hæm and protein takes place. For example, they thought that when the hæmochromogen formed is oxidised hæm reappears.

acids, amines, ammonia, hydrazine hydrate, pyridine<sup>(9)</sup>, nicotine, pyrrol and other nitrogen bases (though probably not all) can be used instead of globin. All these artificial hæmochromogens have similar but not identical properties, *e.g.* taking globin-hæmochromogen as zero, nicotine-hæmochromogen is 3.5 Å. and glycocoll-hæmochromogen 21.5 Å. to the blue, while taking CO globin-hæmochromogen as zero, CO nicotine-hæmochromogen is 16 Å. to the red and CO glycocoll-hæmochromogen 7 Å. to the red. Previous workers have often used hydrazine hydrate or ammonia (plus a reducer) to convert hæm into hæmochromogen without realising that these substances were occupying the position of globin in globin-hæmochromogen.

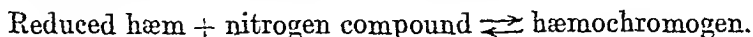
*Helicorubin.* Dhéré and Vegezzi<sup>(14)</sup> have investigated a pigment (helicorubin) present in the bile of certain of those invertebrates containing hæmocyanin in their blood. Helicorubin has an absorption spectrum very similar to that of ordinary globin-hæmochromogen, the main difference being that in helicorubin the two bands are shifted towards the red. Because of its similarity to hæmochromogen, helicorubin has been called a pseudo-hæmochromogen. Dhéré and Vegezzi showed that it is a conjugated protein, but they failed to extend this view to globin-hæmochromogen. The differences these investigators found between helicorubin and globin-hæmochromogen are especially interesting when it is known that globin-hæmochromogen as well as helicorubin is a conjugated protein. Dhéré and Vegezzi showed that the hæm of helicorubin is probably<sup>1</sup> the same as the hæm of globin-hæmochromogen. To show that the two hæms are the same it would be necessary to remove the proteins and then add the same nitrogen compound to the two reduced hæms. If the absorption bands of the resulting hæmochromogens are in the same position and of the same intensity<sup>(12)</sup>, then the hæms of helicorubin and globin-hæmochromogen are identical. It is now clear that this is practically what Dhéré and Vegezzi did, although they did not measure the intensities of the bands. It would appear, then, that the differences between helicorubin and globin-hæmochromogen are caused by their possessing entirely different proteins and that the similarities between them are caused by their possessing the same hæms. This explains why they have absorption bands with the same patterns but located slightly differently. We are now extending some of the experiments of Dhéré and Vegezzi on helicorubin to

<sup>1</sup> Their method was not sufficiently accurate to definitely establish this point. Using the same method ox and sheep hæmoglobins would appear to be identical, whereas we now know they are different.

determine more exactly some of the differences between it and globin hæmochromogen.

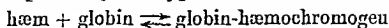
The nature of the linkage between globin and hæm is unknown. The nitrogen group of globin responsible for combination with hæm is not necessarily amino-nitrogen, as has hitherto been assumed. In this connection it has escaped notice that such compounds as pyridine, which have no amino-nitrogen, readily form hæmochromogen with hæm. Possibly the nitrogen of globin that combines with hæm is present as an indol group (as in tryptophane), an imidazol group (histidine) or as a pyrrolidine group (prolin). Finally, the nitrogen in question may be present in one of those forms that Bergmann(3) and others have recently shown occur in peptides and proteins. Perhaps the various forms of nitrogen on the surface of the globin molecule compete for the possession of hæm. The work of Bergmann suggests that the hydrogen-ion concentration may be a deciding factor in such a competition. It will be shown later that the transformation from hæmoglobin to hæmochromogen is reversible and that the relative amounts of these substances present in a solution depends on cH. Perhaps cH exercises its influence on the equilibrium between hæmochromogen and hæmoglobin by affecting the competition of nitrogen groups of globin for hæm. As will be pointed out later, the transformation from hæmoglobin to hæmochromogen may be of an entirely different nature.

II. *Hæmochromogen equilibria.* Although the precise nature of the linkage between hæm and the nitrogen group in hæmochromogen is not known, we have devised some experiments which throw light on the chemical equilibria occurring in hæmochromogen solutions. It would seem as if there is an equilibrium of this kind:



If either hæm or the nitrogen compound is added to such a system the equilibrium should be shifted towards the right and more hæmochromogen formed, while if either hæm or the nitrogen compound is removed from the system the equilibrium should be shifted to the left and hæmochromogen should disappear. Such experiments can be carried out. If to a hæmochromogen solution prepared by the action of caustic soda on reduced hæmoglobin a little hæm is added, the concentration of hæmochromogen will increase. This experiment can easily be done with the Hartridge reversion spectroscope. The absorption band of ammonia-hæmochromogen is about 25 Angström units more to the red than the band of globin-hæmochromogen. If samples of both hæmo-

chromogens are placed before the spectroscope so that light goes through both, the band observed will lie neither in the position of globin-hæmochromogen nor in that of ammonia-hæmochromogen but in an intermediate position. If one of the hæmochromogens is made more concentrated, this intermediate position shifts in the direction of the hæmochromogen whose concentration is increased. For example, if the intermediate position is 15 units from the position of globin-hæmochromogen and the concentration of globin-hæmochromogen is increased, the band will move to a position perhaps only 10 units from the position of globin-hæmochromogen. By observing the shifts in absorption bands under these conditions we can detect and measure changes in the hæmochromogen concentration. Our experiment consists in adding hæm to a hæmochromogen solution and detecting the increase in hæmochromogen concentration by this method. Equal volumes of a dilute solution of hæmochromogen prepared by adding caustic soda and sodium hydro-sulphite to a hæmoglobin solution are measured out. To one sample are added several c.c. of a dilute solution of hæm in NaOH, while to the other sample an equal volume of water is added. Two wedges of the same thickness are filled with the solutions. A third wedge is filled with some of the hæm solution. A fourth wedge is filled with a solution of ammonia-hæmochromogen. The wedges are arranged in two systems: (1) light goes through a wedge containing ammonia-hæmochromogen and through one containing a globin-hæmochromogen solution to which hæm solution has been added; (2) light goes through a wedge with ammonia-hæmochromogen, then through the wedge with globin-hæmochromogen to which water has been added and finally through a wedge containing hæm solution. In both cases the resultant absorption band will be between the ammonia-hæmochromogen and globin-hæmochromogen bands. The only difference in the two systems is that in one hæm is mixed with hæmochromogen while in the other hæm and hæmochromogen are in separate wedges. If the hæm does not react with anything in the hæmochromogen solution to which it has been added, the bands in the two systems will be in exactly the same position. It is actually found that the band in system (1) is nearer the globin-hæmochromogen band than is the band of system (2). Still more striking is the fact that the band of system (1) is definitely blacker than the band of system (2). Consequently, the concentration of globin-hæmochromogen was increased by adding hæm to a globin-hæmochromogen solution. This can be interpreted by the hypothesis that the equilibrium



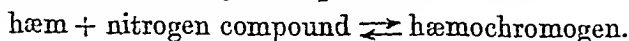
occurs, and that the system is shifted to the right when hæm is added to it.

This experiment throws light on what happens when caustic soda is added to a solution of reduced hæmoglobin. The accepted view is that, as hæmochromogen is formed from hæmoglobin, all the globin is set free from the combination with hæm. We have shown that hæmochromogen itself contains globin and this experiment shows that when hæmochromogen is made from hæmoglobin, the amount of globin set free is very small compared with the total globin present in hæmoglobin. This amount of globin is so small that it can only be accounted for on the hypothesis that globin-hæmochromogen is slightly dissociated.

Further evidence for the existence of the equilibrium is the fact that when hæmochromogen is made from hæm by adding glycoll, ammonia, egg albumin, etc., the amount of the nitrogen compound added must be in excess of the amount of hæmin present if the maximum possible yield of hæmochromogen is to be obtained. That is to say, in the system hæm + nitrogen compound  $\rightleftharpoons$  hæmochromogen, an excess of nitrogen compound is needed to shift the equilibrium to the right. The excess needed varies enormously from substance to substance. Much less globin than egg albumin is required and less egg albumin than ammonia or glycoll. Only a trace of globin will produce more hæmochromogen from a given amount of hæm than will several c.c. of concentrated  $\text{NH}_4\text{OH}$ . Only pyridine and nicotine (of the substances we have tried) even compare in intensity of activity with globin. If it were not for this property of globin—its tremendous affinity for hæm—the existence of globin-hæmochromogen, and therefore probably of hæmoglobin also, would be impossible without the presence of an enormous excess of globin. The hæmoglobin molecule would hardly be “fit” biologically if it depended for its existence on an environment teeming with globin molecules.

If equilibria such as reduced hæm + glycoll  $\rightleftharpoons$  glycoll hæmochromogen and reduced hæm +  $\text{NH}_3$   $\rightleftharpoons$   $\text{NH}_3$  hæmochromogen exist, it would be expected that by adding enough  $\text{NH}_3$  to glycoll-hæmochromogen the glycoll would be displaced and  $\text{NH}_3$ -hæmochromogen formed instead of glycoll-hæmochromogen. This experiment can readily be done in front of the spectroscope and the changes followed by shifts in the absorption bands.

III. *The two forms of hæmochromogen.* The reaction occurring in the formation of a hæmochromogen from hæm seems to be more complicated than would be represented by the equation





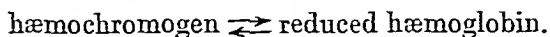
It would seem as if a compound were formed intermediate between hæm and the ordinary hæmochromogen. When just a little egg albumin (and this holds for the other nitrogen compounds too) is added to hæm a compound with the characteristic hæmochromogen spectrum is formed. As more albumin is added the position of this band shifts towards the red end of the spectrum until it finally reaches a position about 25 Angström units from the original position. Further addition of the albumin does not shift the band. The shift cannot be due simply to a mixture of hæm and hæmochromogen because in a mixture of hæm and hæmochromogen the former does not shift the band of the latter. A more satisfactory explanation is that there are two egg albumin-hæmochromogens and that the amount of the egg albumin added determines the relative proportions in which the two will be present. If a relatively small amount of albumin is added to hæm one form of hæmochromogen ( $\alpha$  albumin-hæmochromogen) is observed, but as more albumin is added the other form of hæmochromogen ( $\beta$  albumin-hæmochromogen) appears, the absorption bands of the two forms mixing to give a band in between the bands of the two forms taken separately. Finally, after the addition of enough albumin the band shifts no more, indicating that practically only the  $\beta$  form of hæmochromogen is present. As the absorption band shifts the solution becomes less and less cloudy until it is finally clear, showing that  $\beta$  hæmochromogen is more soluble than  $\alpha$  hæmochromogen. It is interesting to note that the band of  $\beta$  globin-hæmochromogen is to the red of that of the  $\alpha$  form, whereas in the pyridine hæmochromogens the  $\beta$  form has its band at least 40 Å. to the blue of the band of the  $\alpha$  form. Evidence has been given that albumin-hæmochromogen is in equilibrium with hæm and albumin, that there are really two albumin-hæmochromogens, that they have slightly different absorption bands and very different solubilities, and finally that the concentration of egg albumin in solution determines the relative amounts of the two hæmochromogens obtained.

In the case of  $\text{NH}_3$ -hæmochromogen all this can be shown by removing  $\text{NH}_3$  gradually as well as by adding it gradually. An  $\text{NH}_3$ -hæmochromogen solution is put into a tonometer which is gradually evacuated and the changes followed spectroscopically. As the  $\text{NH}_3$  bubbles off the equilibrium between  $\text{NH}_3$ , hæm and hæmochromogen is disturbed. The absorption band gradually shifts towards the red, showing that  $\alpha$   $\text{NH}_3$ -hæmochromogen is being formed at the expense of  $\beta$   $\text{NH}_3$ -hæmochromogen. As still more  $\text{NH}_3$  is removed the band of  $\alpha$  becomes fainter and the spectrum of reduced hæm appears. At the same time

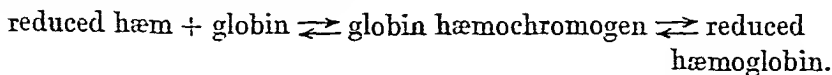
that these optical changes are occurring a red precipitate forms because  $\alpha$  hæmochromogen is less soluble than  $\beta$  hæmochromogen and reduced hæm is less soluble than  $\alpha$  hæmochromogen.

An opportunity to apply these views on hæmoglobin and hæmochromogen is offered by Schulz's well-known method for preparing globin. A few drops of dilute HCl are added to a dilute aqueous solution of hæmoglobin to convert the latter into acid hæmatin. Ether and alcohol in the proper proportions are added and the mixture shaken. The alcohol-water phase loses its colour. It contains globin only. The alcohol-ether phase contains the coloured component of hæmoglobin. The HCl does not break down the hæmoglobin into hæm and globin. Hæm is insoluble in an acid aqueous solution and since no precipitate appears when acid is added to hæmoglobin, no appreciable amount of free hæm can be found. HCl changes hæmoglobin into acid hæmatin—a compound of hæm and globin—and this protein is readily soluble in acid. When alcohol and ether are added to the acid hæmatin solution the equilibrium present ( $\text{hæm} + \text{globin} \rightleftharpoons \text{acid hæmatin}$ ) is disturbed. The hæm goes into the alcohol-ether phase (in which it is very soluble and in which globin is insoluble) thereby colouring it, while the globin remains in the now only slightly-coloured aqueous phase. The hæm so prepared will show the characteristic absorption spectra of hæmatin or hæmochromogen only after globin (or some globin substitute) has been added to it. A considerable separation of hæm can be obtained by Schulz's procedure even without using any alcohol.

IV. *The equilibrium between hæmochromogen and hæmoglobin.* The part played by hæmoglobin in these hæmochromogen equilibria is difficult to determine. The change from hæmoglobin to hæmochromogen is readily reversed and depends merely on changes in the cH. Adding acid or alkali to reduced hæmoglobin converts it into hæmochromogen, while neutralising hæmochromogen turns it into reduced hæmoglobin. This suggests that in a hæmoglobin system there is an equilibrium of the form

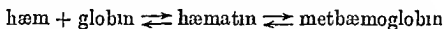


The position of the equilibrium is determined by cH. No hæmochromogen has been detected in neutral solutions of hæmoglobin, but it seems reasonable to expect at least an infinitesimal amount to be present at a cH on either side of which much is present. We now have a series of equilibria of the general nature



Starting from any point in this series of equilibria any other point can easily be reached. The whole series is reversible. Beginning at hæmoglobin a change in the cH will yield hæmochromogen. By means of Schulz's experiment one can proceed from hæmochromogen to hæm and globin. Beginning with hæm and globin, hæmochromogen can be made simply by putting reduced hæm and globin together in alkaline solution. Neutralising this solution turns the hæmochromogen into reduced hæmoglobin<sup>1</sup>. If the components of hæmoglobin exist side by side in nature the synthesis of either hæmochromogen or hæmoglobin will occur, the cH deciding which one. Hæmoglobin synthesised from hæm and globin forms oxyhæmoglobin with an  $\alpha$  band in exactly the same position as that of the oxyhæmoglobin from which the globin was prepared.

A similar series of equilibria exists for methæmoglobin



It might be objected that in a hæmoglobin solution no hæmochromogen could exist because if it did it would be oxidised to hæmatin—an irreversible process which would ultimately turn all the hæmoglobin into hæmatin and thence to methæmoglobin. Just how rapid such a conversion would be is not known. It is known, however, that hæmoglobin does gradually turn into methæmoglobin. This transformation is often due to the acid formed on standing, but even if care is taken to prevent changes in cH hæmoglobin will gradually turn into methæmoglobin. A hæmoglobin solution (containing a little fluoride) was kept at  $-7^{\circ}\text{C}$  for a year. The hæmoglobin turned into methæmoglobin. The cH was about  $10^{-8.2}$ . On the whole the evidence for the hæmochromogen hæmoglobin equilibrium is not conclusive.

It is unknown whether the conversion of hæmochromogen into hæmoglobin is an internal rearrangement of the molecule (possibly of the kind mentioned on p 52) or a polymerisation. A molecular weight determination of hæmochromogen would decide between these alternatives. Hæmoglobin and hæmochromogen may have the same molecular weight or that of hæmoglobin may be some simple multiple of the molecular weight of hæmochromogen. Experiments are now in progress to determine the molecular weights of hæmochromogen and globin<sup>3</sup>.

<sup>1</sup> In this connection cf. Bertin Sans and de Montessier (4).

<sup>2</sup> We want to thank Mr Harold Taylor for the opportunity of carrying out this experiment.

<sup>3</sup> Some preliminary experiments of Adair's to decide this point show that the molecular weight of acid hæmatin (and therefore also of hæmochromogen) is about one quarter that

## PART II. THE RÔLE OF GLOBIN IN HÆMOGLOBIN.

Reduced hæm, a compound with diffuse absorption in the yellow, can combine reversibly with carbon monoxide to form a compound whose spectrum has the general pattern of the CO-hæmoglobin spectrum. But hæm is a biologically impossible gas carrier. It is insoluble, for instance, in water. Before its fundamental ability to combine with a gas can be used in a respiratory pigment almost all its properties must be modified radically. What is the mechanism by which hæm is altered and hæmoglobin formed? Is it simply a matter of tacking on a protein? In other words, has hæmochromogen already the fundamental qualities of hæmoglobin? Will any protein do or must it be globin? Our thesis is this, that from every point of view  $\alpha$  hæmochromogen is a step forward from hæm towards an ideal respiratory pigment,  $\beta$  hæmochromogen a step forward from  $\alpha$  hæmochromogen and hæmoglobin finally from  $\beta$  hæmochromogen.

*Solubility.* Hæmoglobin is very soluble around the neutral point, under physiological conditions. One c.c. of blood can carry about fifty times as much oxygen as one c.c. of water. Reduced hæm, on the contrary, is completely insoluble in water (it does not even colour the water) and for that matter in acid. And it is only sparingly soluble in what biologically speaking is an extremely alkaline solution. Under no conditions therefore could reduced hæm greatly increase the oxygen-carrying power of water, and under the conditions existing in blood, not at all.

The mere addition of protein, any protein, causes a fundamental advance. There are important qualitative changes in solubility. Unlike hæm, hæmochromogen is an ampholyte<sup>1</sup>. It is only slightly soluble around its isoelectric point, that is, around the neutral point, though of course much more so than the completely insoluble hæm. One can easily prepare an alkaline solution of hæmochromogen with a much greater gas capacity than that of many bloods. Furthermore,  $\beta$  hæmochromogen is much more soluble than  $\alpha$  hæmochromogen and globin-hæmochromogen of hæmoglobin. Adair finds the molecular weight of hæmoglobin to be about 68,000 and that of hæmatin to be about 17,000. This proves that hæmoglobin is a polymer of hæmochromogen, four molecules of hæmochromogen polymerising to form one of hæmoglobin. Experiments on the molecular weight of globin are now in progress. Should the molecular weight of globin be 8000 the difference between  $\alpha$  and  $\beta$  hæmochromogen would be clear; in  $\alpha$  hæmochromogen one molecule of globin would be combined with one of hæm, whereas in  $\beta$  hæmochromogen two molecules of globin would be combined with one of hæm.

<sup>1</sup> This is easily shown for the oxide. It is interesting that the oxides of both hæm and hæmochromogen are much more soluble than the corresponding reduced compounds.

gen is much more soluble than the artificial hæmochromogens made from gelatine, globulin, glycocoll or ammonia. The addition of a protein then, and of globin in particular, creates out of hæm a substance which, in great contrast to hæm, is very soluble, although not at a hydrogen-ion concentration possible for body fluids. For that the globin must be combined as it is in hæmoglobin itself. To bring about this change it is not necessary to add or to remove any component of the equilibrium system. Bring the alkaline solution of hæmochromogen to a physiological hydrogen-ion concentration, that is, neutralise it, and the equilibrium is shifted from the water insoluble hæmochromogen to hæmoglobin, the only form soluble enough to carry much oxygen in an almost neutral blood.

*Reversible combination with oxygen.* Hæmoglobin can react with oxygen in two very different ways. It can react to form the oxy-compound found in blood. The oxygen in this case comes off from its combination in an oxygen-free atmosphere, such as exists in the tissues. Hæmoglobin can also react with oxygen to form the true oxide(s), methæmoglobin, from which the oxygen cannot be pumped off. Fortunately the oxygen of the air has not a high enough oxidation potential to convert reduced hæmoglobin into methæmoglobin. For that one has to use ferricyanide or some such reagent with a much higher oxidation potential. Although molecular oxygen cannot convert hæmoglobin into its oxide, it does that very thing to hæm or hæmochromogen. To reduce the compounds formed when hæm or hæmochromogen are exposed to the air, one has to use a strong reducing agent, such as  $\text{Na}_2\text{S}_2\text{O}_4$ . Evacuation has no effect. The transformation from hæmochromogen to hæmoglobin then involves the biologically essential increase in the oxidation potential necessary for oxidation. If it did not, the blood would contain not hæmoglobin but methæmoglobin.

In connection with the mechanism of this, some experiments we performed with pyridine are interesting. If crystalline hæmin is added to pure pyridine (a very weak base) a solution is obtained with a spectrum more or less like that of oxyhæmoglobin. On long standing this solution changes until it finally gives a hæmochromogen spectrum. This final hæmochromogen is obtained immediately if the solution is exposed to a vacuum or a reducer is added. The idea at once comes to mind that the combination between pyridine-hæmochromogen and oxygen under these conditions is similar to the loose combination between hæmoglobin and oxygen. But the pyridine-hæmochromogen does not go to the oxy-form on exposure to air or even to pure oxygen. What is remarkable,

however, is that when acid is added the hæmochromogen combines with the oxygen of the air to give the compound with the oxyhæmoglobin-like spectrum and this bound oxygen can easily be pumped off again. Probably the oxygen would combine with the pyridine-hæmochromogen even in neutral solution were the oxygen tension only high enough<sup>1</sup>. Here we have a hæmochromogen which can combine reversibly with oxygen, a compound which, though lacking the hæmoglobin structure, is not oxidised by molecular oxygen. Perhaps the potential needed to oxidise globin-hæmochromogen is greater than needed for hæm. Perhaps a hæmochromogen with some other protein substituted for the globin might resemble the pyridine compound in its behaviour towards oxygen. With globin at any rate, to avoid oxidation by the oxygen of the air, it is essential that the globin be combined as it is in hæmoglobin.

*The influence of hydrogen-ion concentration on the dissociation curve.* Another remarkable fact about pyridine-hæmochromogen is that its affinity for oxygen is increased by the addition of acid. Even more striking is the fact that bubbling CO through neutral pyridine-hæmochromogen produces no change, while CO combines easily with acid pyridine-hæmochromogen. This is altogether surprising, for in the apparently analogous reaction between hæmoglobin and oxygen the situation is reversed. What is biologically important, the carbon dioxide that enters the tissue capillaries tends to drive out the oxygen. The simplest explanation is that the mechanism is the same in both cases, that it is simply a matter of the influence of the hydrogen-ion concentration on the dissociation of a weak acid but that ionised pyridine-hæmochromogen has a smaller affinity for oxygen than has the unionised form. One must keep in mind nevertheless the alternative explanation that the acid acts directly on the pyridine part of the molecule. Redfield and Hurd (13) have just provided some interesting information which is to the point. The hæmocyanin of the squid has less affinity for oxygen the more CO<sub>2</sub> is present. But the hæmocyanin of the horse-shoe crab has a greater affinity for oxygen the more CO<sub>2</sub> present, the reverse of what is true in the oxyhæmoglobin case and the same as in the pyridine hæmochromogen case. It looks as if in the hæmocyanins the nature of the protein decides the direction of the influence of hydrogen-ion concentration, for the copper-containing portions of the molecule are probably the same in both pigments. In the hæm derivatives too, does

<sup>1</sup> We do not know why the oxy-compound is first formed when hæmin is added to pyridine. Cf. the paper of Von Zeynek (17), whose experimental results are different from ours.

the nature of the protein decide the effect of pH on affinity? One would also like to know whether CO-hæm is a stronger acid than hæm, and CO-hæmochromogen than hæmochromogen

*Specificity* The oxygen dissociation curve of any hæmoglobin must be adapted to the special respiratory needs of the animal and indeed two distinct mechanisms for this adaptation are known. The affinity of a concentrated hæmoglobin solution for oxygen is greatly affected by the ions present in the solution, in particular by the hydrogen ions. If the ionic composition of one blood is different from that of another, as is certainly the case, then for this reason alone the dissociation curves cannot all be the same. But, in addition, the hæmoglobins themselves vary, they have different affinities for oxygen even when placed in identical chemical environments. Such specific variations have recently been shown and measured for the hæmoglobins of even closely related animals (1, 10). Sheep's hæmoglobin, whatever ions are present, has 250 times as great an affinity for carbon monoxide as for oxygen, rabbit's hæmoglobin only 150 times as great an affinity. Intimately related to this are the quantitative differences in the spectra of sheep's oxy and carboxy hæmoglobins on the one hand and rabbit's oxy and carboxy hæmoglobins on the other. Thus the  $\alpha$  band of sheep CO hæmoglobin is 5 Å to the blue of that of rabbit CO hæmoglobin. Given the measurements of these spectra one can predict quantitatively from the optical data alone the relative affinities for carbon monoxide and oxygen of sheep's and rabbit's hæmoglobins.

The chemical basis of this specificity must be in the globin part of hæmoglobin for the hæm part is almost certainly invariable. In general it is known that the electron orbits of one atom can be deformed by the electric field of a neighbouring atom. The most convenient way of following this deformation is by measuring the changes in optical properties due to it. But quantitatively related to these changes in optical properties and caused by the same mechanism are the changes in the ordinary chemical properties. In the case of the hæmoglobins, then, the globins deform the hæm molecules and deform them differently. Hence the various hæmoglobins have different spectra and different affinities for oxygen and for carbon monoxide<sup>1</sup>.

A complication is introduced by an observation reported in a previous paper with Barcroft and Olinna (1). Although the various hæmoglobins have different spectra the hæmochromogens and the carboxy-

<sup>1</sup> We intend in a future paper to provide further information on the relations between the spectra and the chemical properties of various hæmoglobins and their derivatives.

hæmochromogens prepared from them all have absorption bands in the same positions. From the general argument that the spectra are a precise measure of deformation, one must conclude that all the hæmochromogens have the same affinity for carbon monoxide. We know, on the other hand, that the hæmochromogens contain different globins or the hæmoglobins could not be different. The hæmochromogens are different compounds because they contain different proteins and therefore one would expect the affinities for carbon monoxide to be different. Both arguments cannot be entirely correct. But what are the facts?

The necessary experiment is to take rabbit and sheep hæmoglobin which have very different affinities for CO and to compare the affinities for CO of the hæmochromogens prepared from them. We did this and found them to be the same. So far as this reaction is concerned the globins are specific only when they are combined as they are in the hæmoglobin molecule. In hæmochromogen slight differences in the globins are not sufficient to influence measurably either the positions of the bands or the affinity for carbon monoxide.

*The temperature coefficient.* The temperature coefficient of the reaction between sheep hæmoglobin and oxygen or carbon monoxide is about four, which is much higher than that of most chemical reactions. The reaction between hæmochromogen and carbon monoxide, on the contrary, has a temperature coefficient of about 1.2, which is much lower than that of most chemical reactions. The peculiar relations of the globin to the rest of the hæmoglobin molecule are thus important in determining the temperature coefficient.

*The hæmoglobin model.* The hæmoglobins and the heliocorubins found in nature have all the same hæm. The small specialised part of the molecule is constant. The big protein part however which controls, and even creates, to so great an extent the properties of these respiratory pigments varies greatly in adaptation to variations in respiratory needs. This particular arrangement is so economical, so natural, that one would expect it to be common in nature. And indeed the work of Willstätter and his school on enzymes has provided us with several examples of the same sort. Pepsin and trypsin, when sufficiently purified, turn out to be identical non-protein substances, having the same optimum pH for their activity. In nature the pure enzymes are combined with unknown but certainly different colloids. It is the differences in these "Begleitstoffe" that cause the differences in the optimum pH for the activities of pepsin and trypsin as we find them in the digestive system<sup>(15)</sup>. A still clearer case is that of the castor bean lipase. The optimum pH for the



lipase in the young bean is different from the pH of its medium. In the course of embryonic development (and this can be imitated *in vitro*) a proteolytic ferment attacks and modifies the protein with which the pure lipase is combined and as a result the optimum pH for the activity of the enzyme becomes identical with that of its medium and fat splitting begins (16). Hæmoglobin is definitely a conjugated protein. The functions of the protein part and of the specialised part are clear. In particular it is clear that it is the protein part which is variable. Perhaps future research will discover the hæmoglobin model to be a common one.

#### TECHNIQUE FOR CO DISSOCIATION CURVES.

The method we devised to measure the affinity for carbon monoxide of Hb or hæmochromogen in dilute solution is this. Into a tonometer of known size (*a*), containing hydrogen and a solution of reduced Hb or hæmochromogen of known CO capacity (*b*), is introduced a definite volume (*c*) of CO. At equilibrium, after rotation in the dark, (*d*) c.c. of CO remain in the saturation atmosphere and this value (*d*) is obtained by direct analysis. The final CO tension in millimetres of mercury then equals the CO left divided by the size of the tonometer (*d/a*) times the barometric pressure. The CO absorbed equals the CO put in minus the CO left (*c-d*). And the percentage saturation equals the CO absorbed divided by the CO capacity of the solution  $\left(\frac{c-d}{b}\right)$ .

What at the same time makes this method possible and presents the main difficulty is the fact that the CO left in the atmosphere at equilibrium is not even detectable by the ordinary methods of gas analysis. We had to titrate the CO with hæmoglobin itself, and then determine spectroscopically the amount of COHb formed. The analysis procedure is this. A slightly alkaline solution of Hb of known CO capacity is placed in a tonometer of known size. The tonometer is simply a round-bottomed milk pasteurisation bottle with a two-way tap stuck through the rubber stopper. The vessel (in practice several at a time) is then evacuated (the Hb being reduced) and filled with oxygen-free hydrogen. We used commercial electrolytic hydrogen which had been passed over hot palladianised asbestos and then stored with special precautions. Reduced Hb remained entirely reduced in this hydrogen even at zero degrees. In other words the oxygen impurity was less than 1 part in 500,000. The analysis tonometer is now connected with pressure tubing to the saturation tonometer and then evacuated together with the connecting tubing. When the tap is turned and the two tonometers brought

into communication with each other a definite fraction of the CO to be analysed passes over into the evacuated tonometer. What the fraction is can be calculated from the sizes of the two containers. The analysis tonometer is then rotated in the dark in a chamber kept at zero degrees<sup>1</sup>. On the basis of approximate knowledge of how the experiment is going to come out, the analysis solution has been made up to have a CO capacity equal to about double the volume of CO that has passed over. So in no case can the analysis solution become more than half saturated. In fact it has to be less than half saturated because at equilibrium a certain amount of CO will be left in the atmosphere. Fortunately this amount can be neglected. Since the affinity of Hb for CO is quadrupled every time the temperature is lowered 10° C., the tension of CO required for 50 p.c. saturation at zero degrees is negligible compared to the tension required for 50 p.c. saturation at the temperatures at which we performed our experiments. The CO left in the analysis atmosphere at equilibrium is less than 1 p.c. of the CO which has been absorbed by the analysis solution.

The problem now is to determine the exact composition of this approximately half-and-half mixture of reduced and carboxyhæmoglobin—a problem which has already been solved by Hartridge. When the mixture is poured out of the tonometer the reduced Hb is oxygenated and the COHb remains unchanged. One might expect that some of the CO would be driven off by the oxygen, but one can show by control experiments that in a short time this does not take place to any measurable extent. At zero degrees hæmoglobin has a 500 times greater affinity for CO than for oxygen. And the CO in solution being at such a low tension diffuses out very slowly indeed. A rise in temperature on the other hand can easily change the CO saturation. It is essential to carry out the whole analysis procedure at zero. We now have a mixture of oxy- and carboxy-hæmoglobins whose percentage composition can be determined spectroscopically with an accuracy of about 3 p.c.<sup>(11)</sup>. The CO absorbed by the analysis solution, that is the CO which has passed over into the analysis tonometer, equals simply the percentage saturation of the solution times its CO capacity. From the CO that has passed over one can calculate the total CO left in the original saturation atmosphere.

A word about the quantities used. Suppose the original solution to be 50 p.c. saturated at equilibrium and the CO absorbed by it in becoming

<sup>1</sup> We wish to thank Sir William Hardy for his permission to use a cold room of the Cambridge Low Temperature Station.

50 p.c. saturated to be equal to that left in the atmosphere. Suppose further the original CO content of the atmosphere and the CO capacity of the solution to be known with absolute accuracy. Then an error of 3 p.c. in analysing the CO left in the atmosphere would result in 3 p.c. error in calculating the amount absorbed. Suppose however the amount absorbed to be ten times that left in the atmosphere, as was usually the case in our experiments. Then an analysis error of 3 p.c. would result in an error of only 0.3 p.c. in calculating the amount absorbed. The percentage saturation can therefore be determined with considerable accuracy, although the final tension is not known with nearly the same precision. This is important as we shall see in the calculation of the equilibrium constant.

The CO capacity of the Hb solution is obtained by a similar procedure. As a matter of convenience, though not of necessity, the capacity of a concentrated solution is first measured approximately with the Barcroft apparatus. Then a definite volume of the solution after it has been reduced by evacuation or with a reducer is exposed, preferably at zero, to a volume of CO equal to the CO capacity of the solution plus what is needed to provide the tension to saturate the Hb with CO completely. After saturation the CO left in the atmosphere is analysed with Hb. With concentrated solutions and a saturation temperature of zero it is easy to make the CO absorbed many times greater than the CO left to be analysed. As a result the CO capacity can be determined to within 0.2 p.c. Hæmochromogen prepared by hydrolysis of Hb we found to have the same capacity as that of the Hb from which it was prepared. The original CO content of the atmosphere (*c*) is also known to 0.2 p.c. So that, taking the experiment as a whole, the error in the saturation may be 1 p.c., in the tension at the worst 5 p.c.

The advantage of this method is that it can be used for hæmochromogen as well as for Hb, for dilute solutions as well as for concentrated ones. And in case of necessity one could work with very small quantities of material, with, say, much less per determination than what corresponds to 0.01 c.c. of blood. The method is laborious when used for a single experiment, though not at all so when used for a series of experiments, for a single known CO mixture lasts indefinitely and the analysis solution will keep for a long time.

We first used this procedure to obtain a complete CO dissociation curve of hæmochromogen<sup>1</sup>. Ox blood was laked, "hydrolysed" with alkali, diluted some twenty times, and reduced with an excess of

<sup>1</sup> A brief report of this was presented to the Physiological Society last May (2).

$\text{Na}_2\text{S}_2\text{O}_4$ . The hæmochromogen must have been completely ionised. As was to be expected, the dissociation curve turned out to be a rectangular

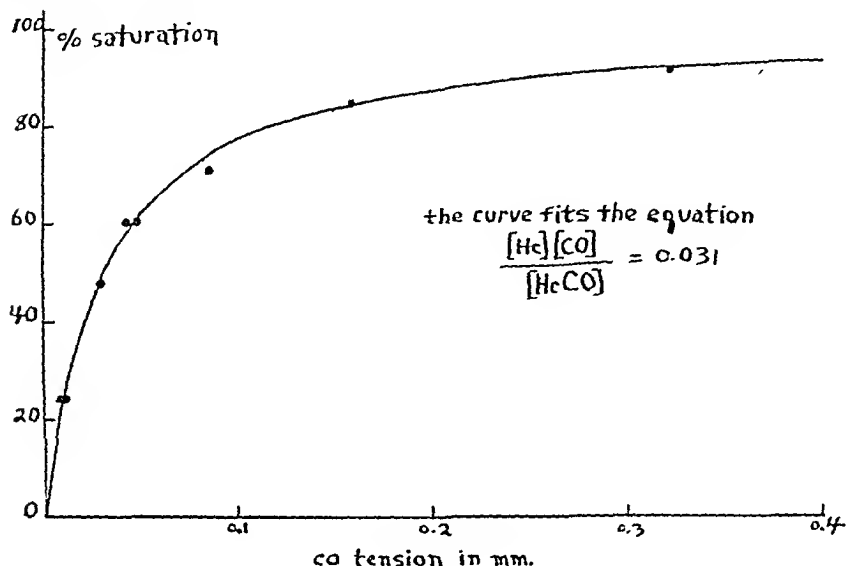


Fig. 1.

hyperbola(2) (cf. Fig. 1). In other words the reaction between hæmochromogen and CO obeys the simple mass law equation

$$\frac{[\text{Hc}][\text{CO}]}{[\text{HcCO}]} = K = 0.031 \text{ mm. at } 37.5^\circ \text{ C.}$$

Furthermore the value of K here is of the same order as for the reaction between Hb and CO.

One can see from the equation the advantage of having the experimental error concentrated on the measurement of the CO tension. An error of 5 p.c. in CO means an error of 5 p.c. in K. An error of 5 p.c. CO-hæmochromogen means first an error of 5 p.c. in hæmochromogen which is simply 100 p.c. CO-hæmochromogen and hence an error of at least 25 p.c. in the fraction. Since the mass law holds, one can determine K by getting any one point on the dissociation curve. We determined K for rabbit and sheep hæmochromogens and found them to be the same.

It is a long way, then, from hæm with its rare ability to combine loosely with a gas to hæmoglobin with its wealth of biologically invaluable properties. And what is perhaps remarkable above all else, it is apparently a single mechanism which produces that great variety of necessary changes which convert hæm from an interesting chemical into a respiratory pigment, a single mechanism which converts an insoluble

iron compound into a substance which can form a 30 p.c. solution and at the same time changes the oxidation potential needed for true oxidation so that the tissues can easily take the oxygen from its carrier.

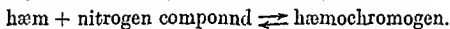
Hæmoglobin is not merely an organic compound perhaps a bit more complicated than the rest. It is a biological molecule. It has structure, just as an organism has structure. It has organisation, just as an organism has organisation. Destroy that structure. What is left has the same chemical composition as at the start. But all the biologically valuable qualities have disappeared. Hæmochromogen, despite its iron and its pyrrol and its protein, is dead and useless.

#### SUMMARY.

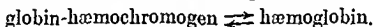
(1) There are many hæmochromogens, each one consisting of an iron-pyrrol molecule (hæm) combined with a nitrogen compound (*e.g.* a protein or amine).

(2) The hæmochromogen prepared by the action of alkali on hæmoglobin contains globin.

(3) In any hæmochromogen there is an equilibrium:



(4) Evidence has been given for the equilibrium:



(5) Information about the influence of globin on the properties of hæmoglobin has been obtained by comparing hæm, the hæmochromogens and hæmoglobin in respect to: (*a*) solubility; (*b*) loose combination with carbon monoxide and oxygen; (*c*) influence of pH on the combination; (*d*) influence of temperature on the combination; (*e*) specificity.

(6) A method has been described for obtaining the CO dissociation curve of hæmochromogen and hæmoglobin in dilute or concentrated solutions.

We desire to express our thanks to Mr Barcroft for his kindness and encouragement to us.

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## THE EFFECT OF PITUITRIN ON THE FATTY ACID OF THE LIVER. BY R. COOPE AND E. N. CHAMBERLAIN<sup>1</sup>.

*(From the Department of Biochemistry, University of Liverpool.)*

THE work described in this paper was undertaken to investigate some of the factors affecting the mobilisation of fat from the depôts and its transference to the liver, and the influence upon it of certain of the internal secretions.

The possibility that extract of the posterior lobe of the pituitary gland might produce a fatty infiltration of the liver was suggested by the clinical association of pituitary disease with striking anomalies of fat metabolism, as in Fröhlich's syndrome; and by physiological facts, such as the increased activity of the gland in the later stages of pregnancy, at which time a definite fatty infiltration of the liver had been found by Coope and Mottram (1914).

Mottram (1909), and Coope and Mottram (1914), having shown that the fat content of the liver was very constant in rabbits, the experiments were confined almost entirely to these animals, usually weighing from  $1\frac{1}{2}$  to 2 kgs. Pregnant and lactating animals were discarded. The rabbits had been kept singly in cages, usually for not less than a month, and were fed on a constant and liberal diet of oats, bran and green vegetables. The preparation used was mainly Parke Davies and Co.'s "pituitrin," though in a few experiments other preparations were employed. It was noted that injections of even 4 c.c. of pituitrin had no appreciable ill-effect either on the general health, or on appetite. This was important as excluding the possibility that any fatty infiltration of the liver obtained was due to hunger.

The pituitrin was injected subcutaneously into the flank. At first the dosage and times of injection were tentative, small repeated doses being given in the earlier experiments, usually within the 24 hours before death. Later, the effect of single, larger doses (3 or 4 c.c.) given at varying intervals before death, was studied, and a "time curve" was plotted. The pituitrin was usually mixed with 5 or 6 c.c. of a warmed saturated solution of gum arabic, which was found to prevent marked local reaction, apt to occur with injection of 3 or 4 c.c. of pituitrin alone. It was

<sup>1</sup> Who received a part-time grant from the Medical Research Council.

thought, too, that the gum would spread the absorption of the extract over a longer period.

The animal was weighed just before death. It was killed by a blow on the back of the head. The liver was quickly excised, weighed after removal of the gall bladder and obvious connective tissue, and minced by a special mincer into a fine cream, which was then well mixed in order that the two aliquot samples taken might be truly representative of the whole liver. In some of the earlier experiments, neglect of this precaution, with a view to saving time or material, resulted in discrepancies in the final percentages of fatty acid, which although small were outside the limits of experimental error. Differences in the amount of fat in different parts of the liver have previously been pointed out by Dowler and Mottram (1918). Only the pure non-volatile fatty acids were estimated, Mottram's (1910) method being used for the purpose. Small pieces of the liver were taken and examined histologically.

A sample of blood was taken from the heart immediately after death—some was used for an estimation of sugar by the Folin-Wu method, and the rest was oxalated and centrifuged for naked eye evidence of lipæmia. Urine was withdrawn from the bladder, and tested for sugar and ketone bodies.

*Control rabbits.* The fatty acid content of the liver was estimated in six control rabbits (Table I A). The mean content was 2.86 p.c. of fresh liver. The figure agrees quite closely with those of earlier work, as for example the "normals" in the pregnancy experiments previously mentioned, where the mean in six animals was 2.62 p.c. It is justifiable, therefore, to regard a percentage higher than 3 as an abnormal fat content of the liver under the conditions of feeding and management described.

*Rabbits injected with pituitrin.* The results with pituitrin are given also in Table I. As the size of the liver varies considerably even in animals of the same body weight, both body weight and total weight of the liver were taken, and the ratios of liver fatty acid to body weight, and to  $(\text{body weight})^{2/3}$  estimated. No obvious relation between these ratios and the percentages of fatty acid in the liver was found. The averages of these estimations are given in Table II, and show that even had the factors of body weight or of surface area to be taken into consideration, the above-mentioned ratios would not contradict the conclusions drawn from the percentages.

The experiments with rabbits given pituitrin with gum not more than 24 hours before death (Table I B a) form the only consistent series



TABLE I Percentage of fatty acid in liver of rabbit

A Controls			
Exp		p c	
1		2 11	
2		2 59	
3		2 70	
4		3 02	
5		3 14	
6		3 59	
Mean		2 86	

B Pituitrin injected in one dose of 4 c c, except as mentioned			
a Injected with gum			
Exp	Hours of action	p c	
7	10	2 52	
8	18	3 94	
9	18	4 11	
10	24	4 12	
11	12	4 53	
12	16½	5 82	
13	10	6 25	
14	18	6 49	
15	15½	7 25	
16*	15½	8 36	
17*	30	2 45	
18	41	3 00	
* 3 c c injected			
b Injected without gum			
19	24	3 03	
20*	19	3 40	
21,	6½	4 88	
* 3 c c injected † 5 c c injected			
c Rabbit fed previously on carrots			
22	15½	3 72	
23	18½	3 69	
d Pituitrin boiled for 6 hours, and injected with gum			
24	16½	4 56	
e Pituitrin boiled with gum for 1½ hours			
25	16	3 41	

C Pituitrin injected in successive doses			
Exp	Doses	Hours from injection	p c
		first last	
26	3 of 3 c c	48 18½	2 78
27	3 of 2 c c	19 2	5 28
Without gum			
28	4 of ½-1 c c	23 2	3 80
29	3 of ½-1 c c	5 2½	4 75
30	3 of 1 c c	26 2½	5 19

for which the mean results can fairly be compared with the normal mean, shown in Table I A

TABLE II Mean results compared

	F A in liver p.c.	Total liver F A (g)	Liver F A Body weight	Liver F A (Body weight) <sup>2/3</sup>
A Controls	2.86	1.51	$8.74 \times 10^{-4}$	$10.6 \times 10^{-5}$
B Single large dose with gum (action 24 hours or less)	5.34	3.17	18.6	22.8

It is seen that in the "pituitrised" animals, the percentage of liver fatty acid is approximately double that of the controls. Even if we lump together all the 24 experiments in which pituitrin was given, with their great differences in time, frequency, amount and conditions of dosage, the effect on the fatty acid content of the liver is still markedly shown by a mean percentage of 150.

In a series of experiments on animals in which it is impossible to

control all disturbing factors, it cannot be expected that each individual experiment will show the same degree of response, or even show the characteristic infiltration at all. Exp. 7 is of interest as illustrating this point. We regard the above experiments, therefore, as amply justifying a conclusion that extracts of posterior lobe of the pituitary body produce a well marked fatty infiltration of the liver.

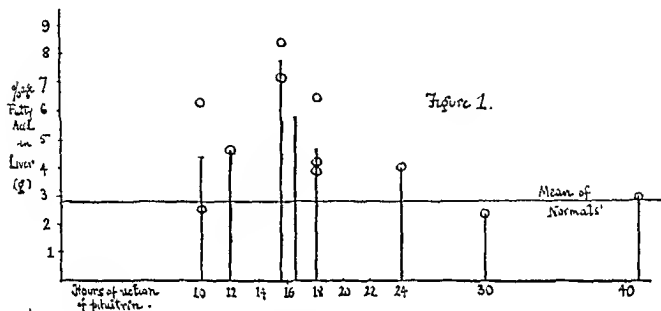
The general appearance of the liver in the "pituitrised" animals was that of a fatty liver. It was softer and paler than the normal dark brown liver; it was frequently mottled, yellowish in tinge, and in some cases almost fluid after mincing. The histological evidence agreed throughout with the chemical analyses. Pieces were frozen in formol-gum, and stained with either Sudan III or Scharlach R. The fat in these infiltrations was remarkable for its equality of distribution. There was only a slight tendency to accentuation at the centre and periphery of the lobule. Specimens of livers showing high percentages of fatty acid were also stained (paraffin sections being prepared for this purpose) with hæmatoxylin and eosin, to demonstrate the cell structure. In no case was there any degeneration either of nucleus or of protoplasm.

*The effect of the medium in which the pituitrin was given.* In the majority of the experiments, the pituitrin was mixed with 5 or 6 c.c. of gum arabic solution before injection; to the rabbits of Table I B *b* the pituitrin was given alone. A comparison of these results, together with Exps. 28, 29 and 30 (Table I C) with those of Table I B *a* (with gum), suggests that the effect was less constant than when gum was added. Two explanations may be offered. It was our experience that the gum prevented any serious local reaction such as was liable to result from a large dose of pituitrin alone and might well interfere with the proper absorption of the drug. It is also possible, though we have no proof of this, that when given with gum, the pituitrin is absorbed more slowly, and its effect is spread over a longer period. It will be shown later that when pituitrin is given with gum, the effect does not pass off until about 24 to 30 hours after the injection. Exps. 21 and 29 strongly suggest that without gum, absorption is more rapid, and that the onset and disappearance of the fatty infiltration of the liver are correspondingly accelerated<sup>1</sup>.

*The period of action of the pituitrin, and the degree of fatty infiltration produced.* In the experiments with single doses, the pituitrin was allowed to act for various periods of time before the animal was killed. Fig. 1

<sup>1</sup> Two later experiments, however, have shown, four hours after injection of 4 c.c. pituitrin with gum, 4.25 p.c. and 6.11 p.c. fatty acid in the liver.

shows the time relations of the liver infiltration when pituitrin was given with gum (3 or 4 c.c. pituitrin in 5 or 6 c.c. of gum arabic solution). Since it is based on comparatively few experiments, it must be regarded as affording at best a rough approximation to the true curve.



Gum arabic solution alone does not produce fatty infiltration of the liver. In Exp. 2, 8 c.c. of the gum solution was injected subcutaneously into an animal which was killed 18 hours later. The fatty acid content of the liver was 2.59 p.c., a normal figure. Further controls are afforded by the experiments with "deactivated" pituitrin given with gum (Table III, Exps. 31, 32 and 33).

*Experiments with "deactivated" pituitrin.* A series of experiments was undertaken to show whether pituitrin which had been "deactivated" produced any fatty infiltration. For this purpose the method of Dale and Dudley (1921) was adopted. They found that both pressor and oxytocic principles were destroyed by boiling for 6 hours with 0.5 p.c. HCl. Previously H. S. Adams (1917) had shown that the oxytocic principle could be destroyed by boiling at 100° C., if the H. ion concentration was of the order  $N \times 10^{-5}$ .

Pituitrin was boiled for 6 hours on a water bath (a reflux condenser being used) with an equal volume of 1 p.c. HCl, and then approximately neutralised with  $N/5$  NaOH. From this preparation, the equivalent of 3 or 4 c.c. of pituitrin was taken, mixed with 5 c.c. of the gum solution, injected into three rabbits in the usual way, and allowed to act for 16 to 17 hours. A preparation treated as above, save for the boiling, was injected into a control animal, and showed that this particular batch of pituitrin produced the usual fatty infiltration. Another sample was

boiled for 6 hours with an equal quantity of distilled water and similarly injected. The results are given in Table III.

TABLE III. "Deactivation" experiments.

Exp.	Dose of pituitrin	Hours of action	Liver F.A. (p.c.)
Control. Active pituitrin			
12	4 c.c.	17½	5.82
Control. Pituitrin boiled, no acid			
24	4 c.c.	16½	4.56
"Deactivation"			
31	3 c.c.	16½	2.50
32	3 c.c.	16½	3.27
33	4 c.c.	17	3.30
Mean			3.02

It is thus clear that the substance which causes the fatty infiltration is either the same as the pressor and oxytocic substances, or is destroyed by the same treatment.

With injection of pituitrin boiled for 1½ hours with gum arabic (Exp. 25, Table I B e) there was for some reason absence of the expected infiltration. Whether this apparent "deactivation" occurs regularly under such treatment must be left undecided.

*Experiments with other tissue extracts.* Further control experiments were made to see if injection of tissue extracts other than pituitrin produced fatty infiltration of the liver. "Antuitrin," a preparation of the anterior lobe of the pituitary gland, prepared by the same firm (Parke Davies and Co.), and by a similar method, was thought specially suitable for the purpose. It was given in single doses of 3 c.c. with gum, in the same way as the pituitrin, and the animal killed 15 to 18 hours later. The results are shown in Table IV.

TABLE IV. Experiments with antuitrin

Exp.	Liver F.A. (p.c.)
34	2.16
35	3.37
36	3.40
Mean	2.98

The figures must be compared with those of Table I B a. It is clear that antuitrin produces no such effect as was obtained with pituitrin. It is true that in Exps. 35 and 36, there is a slight increase of the fatty acid percentage above the normal. In Exp. 35, however, the liver was small for the size of the animal (49 g. for a rabbit of 2 kilos.), and in any case the small increase in either experiment may well be due to inclusion of small amounts of posterior lobe material in the "antuitrin."

Experiments made with other tissue extracts have so far also failed to produce fatty infiltration of the liver. It is hoped to publish an account of these in another paper. The absence of fatty infiltration after injection of "deactivated" pituitrin affords further support to the view that the infiltration is due to some special constituent of the posterior lobe of the pituitary body.

*Blood and urinary results in rabbits* No attempts have been made to estimate the blood sugar during the experimental period, lest the taking of samples should upset the animal, and so prejudice the issue. In samples taken immediately after death, the blood sugar varied so widely both in normal and in pituitrinised animals as to make it clear that nervous or other disturbances deprived the results of all significance. The range of variation was from 90 to 500 mg per 100 c c, yet in no case was there any glycosuria.

Throughout the whole of the experiments with pituitrin, the only one in which a lipæmia was observable by the naked eye was rabbit 13. This was also the only case in which there was excess of acetone in the urine (a trace, by Rothera's test). Sugar was always absent in the urine removed from the bladder at death.

*Experiments with rats* Table V shows, although the experiments are few in number, that under appropriate conditions, pituitrin produces the same effect in rats as in rabbits, the dose necessary, relative to the surface area, appears however to be much larger.

TABLE V Experiments with rats

Exp	No of rats	Total body weight (g)	Dose of pituitrin	Hours of action of pituitrin	% fat in liver
37	3 (♂)	520	0	—	3.14
38	3 (♂)	250	1 c c each	5	1.38
39	3 (♂)	—	1 c c	10	2.62
40	4 (♂)	—	1 c c each with gum	24	2.77
41	4 (♀)	722	2 c c " "	15½	4.74
42	4 (♂)	490	2 c c " "	15½	4.46

Three or four rats were used for each experiment, and the livers minced together to give enough material for accurate estimation. With doses of 1 c c of pituitrin, whether alone or with gum, no infiltration occurred. When the dose was increased to 2 c c per rat, and given with gum, fatty infiltration of the liver was found both histologically and chemically in 15½ hours. It should be added that one rat, not included in the above experiments, died 20 minutes after injection of 2 c c of pituitrin.

*The effect of carrot feeding on the pituitrin effect in rabbits* Two ex-

periments were made on rabbits (Table I B e, Exps. 22 and 23) which had previously received a high carbohydrate diet. Rabbit 23 was fed for nine days on carrots only, and then injected with 4 c.c. of pituitrin with gum. During the actual experimental period it received the ordinary diet. Eighteen hours after the injection, the liver contained 3.69 p.c. of fatty acid, as against 3.96, 4.11 and 6.49 p.c. in the comparable experiments 8, 9 and 14 respectively. Rabbit 22 was fed for 14 days before death on the usual diet with the addition of as much carrot as it would eat; 15½ hours after a similar injection of 4 c.c., the liver contained 3.72 p.c. of fatty acid, as against 7.25 and 8.36 p.c. in the comparable experiments 15 and 16 (Table I B a). In both experiments, therefore, the fat content, although increased, was considerably less than in the rabbits injected with similar doses but without previous feeding with carrots. The glycogen of the liver was estimated only in Exp. 22, and was found by Pflüger's method to be 2.52 p.c.

### *Discussion.*

Carraro (1908) found that repeated injections of pituitary extract produced actual necrosis of the liver cells, with "fatty degeneration." In our own experiments there was nothing to suggest damage to the liver cell, even when the fatty acid content was as high as 8 p.c. Moreover, the disappearance of the pituitrin effect within about 30 hours indicates that the accumulation of fat was a physiological process.

There is much to suggest that increased secretion of pituitrin in the living animal leads to increased transfer of fat from dépôts to liver, and that deficient secretion results in accumulation of fat in the dépôts. Thus it is well known that in the later stages of pregnancy, both in man and other mammalia, there is increased activity of the pituitary gland, often associated with a definite hyperplasia; and Dixon and Marshall (1925) have recently shown in experimental animals that the cerebrospinal fluid contains an increasing amount of the oxytocic constituent of pituitary secretion in the later stages of pregnancy. Coope and Mottram (1914) had also found with rabbits that an infiltration of the liver with dépôt fat occurred shortly before parturition. Conversely, the massive accumulation of dépôt fat is one of the most characteristic features of Fröhlich's syndrome, a condition associated with destructive lesions of the gland, and therefore presumably with deficiency of posterior lobe secretion.

Although the two experiments with carrot-fed rabbits appear at first sight to support the view of Rosenfeld (1895, 1903) that accumulation of fat and of glycogen respectively in the liver are mutually antagonistic,

it should be realised that this view was based entirely on observations of highly pathological livers, and may fail to establish itself as a general proposition<sup>1</sup>. So far as the absence of glycosuria and hyperglycemia can justify any inference concerning the glycogen content of the liver, there was nothing to suggest that the pituitrin fat-liver had been depleted of glycogen. In our experiments glycosuria was never found, and Burn (1923) has shown that the prevalent view that pituitrin causes hyperglycemia is incorrect. Further experiments on this aspect of the problem are in progress.

#### SUMMARY.

1. Injection of extract of posterior lobe of the pituitary body into rabbits and rats is followed by a well-marked increase in the amount of fatty acid in the liver.

2. The time relations of this effect are more consistent if the extract is given with gum arabic solution.

3. The fatty acid infiltration after pituitrin with gum reached its maximum between 10 and 15 hours, and disappeared before the 30th hour.

4. The infiltration does not occur if the pituitrin has been previously submitted to such treatment as destroys its oxytocic and pressor constituents.

5. Control experiments with extracts of other tissues have, up to the present, failed to produce fatty infiltration of the liver.

We wish to express grateful acknowledgment for much helpful advice given by Prof. Ramsden throughout this investigation.

We are indebted also to the Medical Research Council for a grant covering part of the expenses.

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<sup>1</sup> In this connection see Mottram, *this Journ.* 33. 310, 311. 1909; and Mottram and Dowler, *ibid.* 52. 173. 1918.

## APPENDIX. Body weight and liver weight of rabbits.

The figures which follow represent—number of experiment (in italics); body weight in grams; liver weight in grams.

*Table I A.* 1, 1420, 61·65; 2, 1427, 40·38; 3, 1980, 57·41; 4, 2020, 58·10; 5, 1922, 39·40; 6, 1720, 35·82.

*Table I B a.* 7, 1470, 72·63; 8, 1830, 73·71; 9, 2020, 105·90; 10, 1420, 48·56; 11, 2020, 61·47; 12, 1836, 42·10; 13, 2480, 78·90; 14, 1930, 62·23; 15, 1320, 49·62; 16, 2110, 68·40; 17, 7, 81·40; 18, 1560, 41·72.

*Table I B b.* 19, 1750, 57·71; 20, 1310, 42·70; 21, 2620, 73·84.

*Table I B c.* 22, 2030, 75·60; 23, 1480, 48·87. *d*, 24, 2541, 81·40. *e*, 25, 2160, 63·65.

*Table I C.* 26, 1310, 42·36; 27, 1820, 63·48; 28, 2750, 79·55; 29, 1182, 39·80; 30, 2002, 97·80.

*Table III.* 31, 1880, 56·27; 32, 1810, 46·26; 33, 1710, 57·75.

*Table IV.* 34, 1920, 84·40; 35, 1975, 48·80; 36, 1135, 37·87.



THE INFLUENCE OF THE SPLEEN IN CARBON MONOXIDE POISONING BY J BARCROFT, C D. MURRAY, D ORAHOVATS<sup>1</sup>, J SANDS<sup>1</sup> AND R WEISS<sup>1</sup>

*(From the Physiological Laboratory, Cambridge)*

THE frequency with which the operation of splenectomy has been performed, without apparently harmful results, has led to the conception on the part of many that the organism can get on as well without a spleen as with one. It was a matter of some curiosity to us to review this conception in the light of recent evidence which goes to show that the spleen is, among other things, a reservoir of red blood corpuscles held in reserve to meet such emergencies as demand an increase in the quantity of hæmoglobin present in circulation. On such a theory, were the organism presented with an issue which made its life depend on the amount of hæmoglobin it could produce, a splenectomised animal should die, when an animal possessed of a spleen should survive. Such an emergency would be a gradual hæmorrhage for it has been shown that if a cat be bled to the extent of 60 c c in an hour, the spleen will contribute 20 c c of fluid which is probably not less rich in red corpuscles than is the blood itself. The actual loss to the circulation would therefore only be 40 c c<sup>2</sup>, which loss proved fatal. Had the cat had no spleen, the presumption is that it would have died when it had lost 40 c c. Hæmorrhage is less susceptible of exact experiment than is carbon monoxide poisoning. We therefore determined to ascertain whether the animals possessed of a spleen survived those from which the spleen had been removed when both were exposed simultaneously to CO poisoning.

Two series of experiments were performed, in both cases on guinea-pigs. In series I the animals fell into three categories (a) splenectomised, (b) operated controls, in which the abdomen had been opened and some omentum or pancreas removed in the vicinity of the spleen, (c) "normal controls" in which no operation was performed. The operations were carried out under ether four days before the animals were exposed to carbon monoxide, except in one experiment in which the time was three days. We found an ether chloroform mixture unsuitable as an anæsthetic, the guinea pigs developed lung trouble, in some cases fatal, with ether.

<sup>1</sup> Travelling Fellows of the Rockefeller Foundation

This statement is based on radiographic work at present in progress

the tissues remained normal. The general course of an experiment in series I may be followed from Fig. 1. In this case nine guinea-pigs were

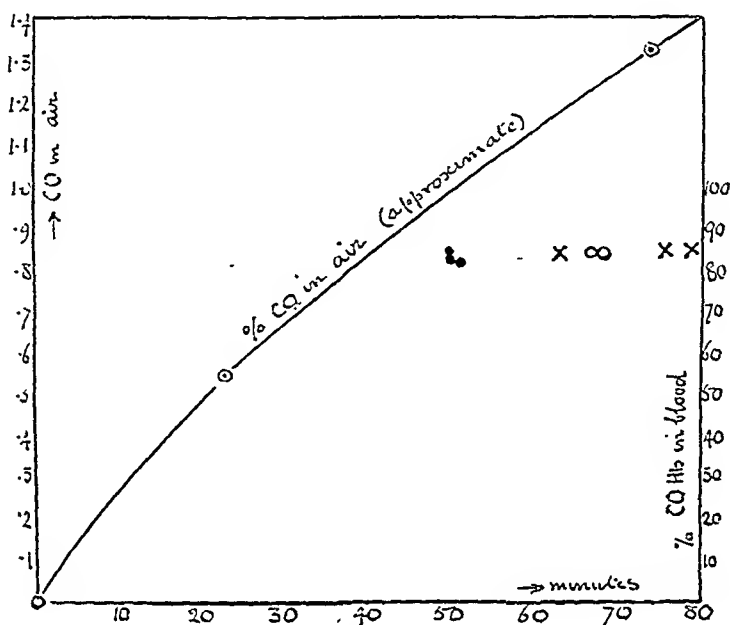


Fig. 1.

exposed, three of each category—one of the operated controls was found post-mortem to be pregnant and was discarded, as were other pregnant animals (except as stated on p. 84). Eight therefore are taken account of in the figure. The animals were more or less of the same size, as judged by the eye, no account was taken of sex. The guinea-pigs were placed in a chamber of 10 cubic metres capacity. Two gas taps in the chamber were turned on and a fan set going to keep the air thoroughly stirred. The CO in the air of the chamber was determined at intervals of about 20 minutes. The guinea-pigs were in wooden trays about 20 inches from the ground and were mixed together so that the observer who was outside the chamber was usually unaware into which category any particular guinea-pig fell. In some of the experiments we were able to secure an observer who was quite unaware of the object of the experiment. The duty of the "observer" was to note the time at which each guinea-pig died. Samples of the air of the chamber were taken for analysis, usually at intervals of about 20 minutes. These could be obtained without entering the chamber. In Fig. 1 the ordinates on the left give the percentage of CO in the chamber air and refers to the diagonal line. The abscissa is the time in minutes

from the turning on of the gas taps. In this particular experiment, which lasted 80 minutes, the final concentration of carbon monoxide in the chamber was 1.4 p.c. The ordinates on the right give the percentage saturation of the blood in each animal at death and refer to the dots, circles and crosses. These dots, circles and crosses refer to animals and are placed above the times at which they died. Reading from left to right, three splenectomised guinea-pigs (●) died 50, 50 and 51 minutes respectively after the gas was turned on. Their bloods contained 83.5, 85 and 83 p.c. of CO respectively. The next animal to die was a normal (×) after 63 minutes' exposure, the next an "operated control" (○) after the experiment had run for 67 minutes, and so on. Thus all the splenectomised animals died before any of the controls, and the operated controls and the normal controls were mixed up in the matter of their longevity. This experiment is typical save for the fact that the three splenectomised animals died within a minute of one another. Usually their deaths, as that of the other categories, were more spread out.

Six such experiments were performed, and for statistical purposes they are treated in the following way. In each experiment the average longevity of the normals in the chamber is calculated and called 100, the longevity of each animal is then calculated as a percentage of that. In the experiment under reserve, for instance, the longevities of the three normals were respectively 63, 76 and 79 minutes. The average longevity of the normals is therefore 72.7 minutes. The individuals are:

	Actual longevity (mins.)	Percentage longevity (mins.)	Mins.
Splenectomised	{ 50	69	69
	{ 50	69	
	{ 51	70	
Operated controls	{ 67	92	93
	{ 68	93	
Normals	{ 63	88	100
	{ 76	104	
	{ 79	108	

Treating each experiment in this way, the results of the whole six become comparable and may be summarised very shortly.

	Normals	Operated controls	Splenectomised
Number of observations	16	15	16
Mean longevity in chamber ( $\bar{x}$ )	100	93.2	72.1
Standard deviation ( $\sigma$ )	8.14	13.02	17.86
Standard error of mean $\sigma/\sqrt{n}$	2.03	3.36	4.46

The question of course arises: are these differences between the categories significant? Taking the categories in pairs and comparing the normals with the operated controls, we find that difference of the means

is only 1.73 times the standard error of their difference  $\left(\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}\right)$ . No significance can therefore be attached to the difference between the normals and the operated controls; but as between the normals and the splenectomised animals the standard error of their difference is 5.68 times the difference of the means and 3.77 times as between the operated controls and the splenectomised; both of which are significant.

Working from the above data, it appears that the probability of the figures as between the normal and the operated animals, coming as they do by chance, is about 25 : 1 against and is of doubtful significance, but that corresponding probabilities as between the normal and the splenectomised and as between the operated controls and the splenectomised are respectively less than 1 in a 100,000,000 and 1 in 100,000, and may be regarded as of significance.

Although from the statistical point of view the above differences are satisfactory, we determined to carry out a control of a purely experimental kind. We therefore performed two experiments in which the gas used was not carbon monoxide, but one which kills the animal for a reason which is quite unconnected with the amount of hæmoglobin in its system. Such a substance is hydrocyanic acid. The animals normal and splenectomised were as in the experiment already described: the HCN was run slowly into the chamber from a burette situated outside. The concentration of gas was presumed to be that caused by the complete evaporation of the hydrocyanic acid measured as a liquid. The experiments lasted a somewhat shorter time than the carbon monoxide ones—about 40 minutes.

In one such experiment the percentage times were as follows:

Normals	69, 116, 116.	Mean 100
Splenectomised	85, 105, 131.	Mean 107

Taking the two experiments together, the mean percentage time both of the normals and the splenectomised animals came out at 100. There was no tendency, so far as could be observed, for the splenectomised animals to die before the normals.

Reverting to the coal gas series, only in one experiment did a splenectomised animal survive animals with spleens. This animal was particularly large—a circumstance which first drew our attention to the influence of size. As all the animals had been weighed after death, we were able, on the assumption that the gas had run at the same rate in all experiments, an assumption which we know to be only very approximate, to plot the time of death against the weight of the animals for the whole six experiments. The result was so interesting that we determined a second series of experiments in which much greater care was taken to have the categories of approximately the same weight. Also in this series

we had operated controls of a different character. We used female guinea-pigs throughout, and in the case of the operated controls one horn of the uterus was removed, thus depriving the animal of a mass of tissue more comparable to the spleen in size and importance, than is a piece of omentum. Otherwise the experiments were carried out as in series I.

Three experiments in this series were performed. Of these Fig 2 is typical. Here the longevity in the chamber (abscissa) is plotted against the weight of the guinea-pig.

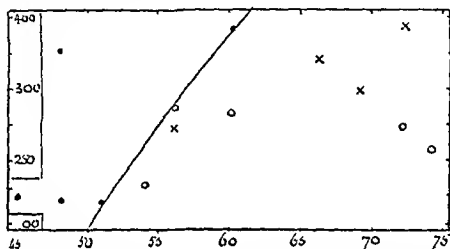


Fig 2 Ordinate and weight of guinea pig in grams abscissa=longevity in chamber in minutes, ●=splenectomised, ○=operated controls, x=normals

It will be seen that the figure can be divided by a line into two areas; to the left are segregated all the splenectomised, to the right the normals and operated controls. The latter are scattered about their area quite indiscriminately.

Worked out statistically, the results of series II are as follows:

	Normals	Operated One horn of uterus removed	Splenectomised
Number of observations	11	12	13
Mean longevity in chamber	100	99	75
Standard deviation ( $\sigma$ )	12.53	12.41	12.75
Standard error $\sigma/\sqrt{n}$	3.79	3.54	3.53

The probabilities against the differences observed occurring by chance are, as between the operated controls and the normals, 8/10, as between the normals and the splenectomised, less than 1 in 1,000,000, and as between splenectomised and the hysterectomised, about 1 in 500,000. It would seem quite clear that the earlier deaths of the splenectomised animals as compared either with the operated controls or the normals is not a chance affair and that the mere fact of an operation does not influence the matter.

In the series very particular care was taken to avoid hæmorrhage, and it may be stated with great confidence that the splenectomised

animals had not lost more blood than the operated controls. In two cases<sup>1</sup> it was quite certain that the operated controls had lost more than the splenectomised, but their life in the chamber was not influenced by the loss. They lived as long as the normal animals.

The question naturally arises: How do the various categories compare in the matter of the saturation of the arterial blood with carbon monoxide? The following are the average figures for the percentage saturation of blood at death with CO:

	Splenectomised	Operated controls	Normals
Number of animals	28	26	28
Average percentage of CO in blood at death	81.9	83.5	83.9

In every experiment except one there was a slight, but very slight, tendency for the splenectomised animal to have a lower percentage saturation of CO than the normals, but on the whole it is remarkable how closely the figures agree. The suggestion is that there is a fatal percentage saturation which is approximately the same for all groups but that under the circumstances of the experiment it is reached sooner in the splenectomised than in the normal animals.

### SUMMARY.

1. In an atmosphere which receives a continuous accession of coal gas, guinea-pigs from which the spleens have been removed die sooner than either normal guinea-pigs, those from which omentum has been removed, or those from which one horn of the uterus has been excised.

2. Excision of the spleen four days previously does not prejudice the length of life of guinea-pigs when exposed to an increasing concentration of hydrocyanic acid, a gas which kills by a means quite unconnected with hæmoglobin.

3. The effects of removal of the spleen are not due to hæmorrhage during the operation.

4. Having regard to the fact that in fatal pressure of carbon monoxide the spleen contracts, expelling its contents into the blood, it is suggested that the longer life of the normal animals is due to this accession of hæmoglobin to the blood.

5. The percentage saturation of the blood with CO is almost the same in all the categories of animals.

The expenses of the above research were in part borne by a grant from the Medical Research Council.

<sup>1</sup> These were found to be pregnant.

## THE MECHANISM OF PANCREATIC DIGESTION—THE FUNCTION OF SECRETIN. BY J. MELLANBY.

*(From the Physiological Laboratory, St Thomas's Hospital, London.)*

A SECRETION of pancreatic juice may be evoked by appropriate stimulation of the vagus (Pavlov(1)) or by the action of secretin (Bayliss and Starling(2)). Generally speaking vagal juice is scanty in quantity but rich in proteins and enzymes; secretin juice, on the other hand, is copious in quantity but relatively poor in protein and enzymes. There appears to be nothing common to the mechanisms involved in these two secretory processes since atropine paralyses the secretory fibres of the vagus but has no apparent action on secretin.

The hypothesis of the chemical control of the digestive functions of the pancreas has been accepted by many observers since the discovery of secretin by Bayliss and Starling in 1902. Evidence however has been shown by various members of the Russian school (Kudrewetzki, Babkin, Savitsch) that pancreatic secretion is due, in part, to reflex action by way of the vagus, and that this nervous mechanism affects especially the protein and enzyme content of pancreatic juice. A detailed account of this aspect of pancreatic secretion is given by Babkin(3). There are also certain inherent difficulties in accepting the doctrine of Bayliss and Starling that the digestive functions of the pancreas depend primarily upon the action of acid chyme on prosecretin, and that prosecretin exists only in that situation in which it may be easily acted upon by acid chyme. Among such difficulties may be mentioned the facts of apparently normal nutrition associated with gastrectomy, achlorhydria, or jejunal alimentation. More particularly the experiments of Dodds and Bennett(4) on duodenal feeding in the normal man directly negative the assumption of the chemical control of pancreatic digestion by gastric acidity. Dodds and Bennett deduced from numerous experiments that the alveolar carbon dioxide pressure gives a measure of pancreatic secretion, and by this method found that the direct introduction of a neutral or even alkaline fluid into the duodenum causes an immediate secretion of pancreatic juice.

In order to determine the relative functions of secretin and the vagus nerves in regulating the digestive functions of the pancreas, the quanti-

tative composition of pancreatic juice was determined under a variety of conditions. The results indicate that the vagus controls the enzyme content of pancreatic juice whilst the volume of bicarbonate solution in which these enzymes are contained is determined by the action of secretin.

*Methods.* The animals used in all the experiments were cats anaesthetised by urethane (1.5 gram. per kilo of body weight). A cannula was inserted into the pancreatic duct, and, in experiments involving continuous secretion, the juice was collected in successive quantities of 3 c.c. These portions were analysed for total alkali, trypsinogen, amylase and lipase.

*Alkali.* 1 c.c. of juice was diluted to 25 c.c. with distilled water and titrated against  $\text{H}_2\text{SO}_4$  0.04 *N* using methyl orange as an indicator. The comparative absence of protein from diluted pancreatic juice enables a fairly sharp endpoint to be observed, especially if indicator controls are used.

*Amylase.* 0.2 c.c. of each specimen of juice was added to 2.3 c.c. of 1 p.c. starch containing 0.1 p.c. NaCl and the achromic time observed. The addition of sodium chloride to the starch paste is necessary since cat's pancreatic juice contains less than 0.2 p.c. NaCl, a quantity less than the optimum required for the action of amylase on starch. The amount of amylase in each sample of juice was determined from a curve showing the achromic times of a similar quantity of starch with known quantities of amylase. It is noteworthy that cat's pancreatic juice contains very little amylase.

*Trypsinogen.* The juice was activated by the addition of an optimal quantity of enterokinase and after one hour the capacity of 0.1 c.c. of the activated juice to clot 2 c.c. of calcified milk (milk to which an equal volume of  $\text{CaCl}_2$  0.1 *N* had been added) was determined. The details of this estimation and the reasons for regarding pancreatic rennin as identical with trypsin have been described in a previous paper (5).

*Lipase.* An emulsion of olive oil was made by adding dilute sodium hydroxide to commercial olive oil until the mixture was just alkaline to phenolphthalein. The soap formed during the neutralisation of the fatty acid in the oil facilitated the formation of a permanent emulsion on shaking the oil with water. To 2 c.c. of oil emulsion 0.5 c.c. of fresh pancreatic juice was added and the mixture was incubated at 40° C., the tube being shaken every ten minutes to preserve complete mixing of the oil and juice. After one hour 2.5 c.c. of absolute alcohol was added to the mixture and the amount of alkali required to bring solution back



to a reaction just alkaline to phenolphthalein determined. This quantity of alkali gave a direct measure of the lipase content of the juice. It is important to estimate the lipase when the juice is freshly secreted since lipase is very rapidly destroyed by trypsin, which may spontaneously develop in the juice *in vitro* even without the addition of enterokinase (Mellanby and Woolley(6)).

*The composition of successive portions of pancreatic juice.* Pancreatic juice continuously secreted by a cat under the stimulus of secretin injected into a femoral vein was collected in seven portions. The following figures show the relative quantities of alkali, trypsinogen and amylase in the successive portions. The enzymes are given in terms of arbitrary units obtained from the standard curves. All the figures in this and the following experiments are comparable with one another:

NaHCO <sub>3</sub> N	·128	·124	·134	·128	·132	·128	·124
Trypsinogen	665	640	360	400	230	280	150
Amylase	340	290	160	160	70	70	60

Two facts are evident from the above figures: (1) throughout the experiment the quantity of NaHCO<sub>3</sub> contained in the successive portions of juice remained approximately constant about a mean value of 0·128 N, and (2) the quantities of trypsinogen and amylase continually decreased, in the case of trypsinogen the content of the final fraction being less than one quarter, and of amylase approximately one-sixth of the quantity of enzyme in the first fraction.

*The influence of pilocarpin on the composition of pancreatic juice.* It is evident that the diminution in the enzyme content of successive fractions of pancreatic juice may be due to the exhaustion of the gland under the constant secretin stimulus. This hypothesis, however, does not accord with the histological appearance of the gland at the end of a long period of secretion. Thus a gland obtained from a dog which had secreted 150 c.c. of pancreatic juice under the stimulus of secretin had a typical resting structure. To test the exhaustion hypothesis, five successive fractions (3 c.c.) of pancreatic juice were obtained by means of secretin from a cat, during a period of three hours. At the end of this time secretin containing 2 mgrm. of pilocarpin was used as the pancreatic stimulant and an additional quantity (4 c.c.) of juice collected. The following figures give the analyses of the various fractions of juice:

	Secretin only					Secretin containing 2 mgrm. pilocarpin
NaHCO <sub>3</sub> N	·150	·138	·156	·148	·146	·146
Trypsinogen	1350	1300	1150	1000	800	1300
Amylase	270	210	160	160	60	200

The figures show the facts previously described—the constancy of the bicarbonate and the steady diminution in trypsinogen and amylase content of successive fractions of juice secreted under the stimulus of secretin only. After the injection of pilocarpin the quantity of alkali remained the same as that in the previous fractions of juice, but the trypsinogen and amylase contents were considerably raised. From general considerations it may be assumed that pilocarpin stimulates the secretory nerve endings of the vagus in the pancreas. Therefore the last sample of juice owed its composition to the simultaneous stimulation of the secreting cells of the pancreas by secretin and the vagus. It contained the same quantity of bicarbonate as the portions of juice produced under the influence of secretin only. Its enzyme content was, however, considerably greater than that of the juice secreted immediately before it and approximated to that of the first sample of juice secreted in the experiment. Therefore secretin caused the pancreas to produce a free flow of  $0.15\text{ N. NaHCO}_3$  which carried with it the enzymes contained in the cells of the pancreas. On the other hand, the enzyme content of the juice appeared to be determined by the secretory fibres contained in the vagus nerve. This hypothesis receives support from the observations of Babkin, Rubaschkin and Savitsch(7) on the histological appearances of the pancreas after the injection of secretin and after stimulation of the vagus nerve. They found that in the case of secretin the pancreatic cells after the production of a copious secretion show no sign of fatigue, being still full of fine granules; after vagus stimulation marked cellular changes are apparent. "Under the influence of secretin, water flows through the cells in quantity and one sees in the cells what look like channels of fluid. This current carries out the zymogen granules into the ducts where they can be seen as granules but soon become dissolved. After nerve stimulation, on the contrary, the granules inside the cells undergo a transformation sometimes forming large vacuoles before passing into the duct." This dual hypothesis of the relative functions of the vagus and secretin, brings into line the work of Pavlov on the nervous mechanism of pancreatic secretion with the work of Bayliss and Starling on the chemical control of pancreatic secretion.

*The influence of atropine on the composition of pancreatic juice.* Wertheimer and Lepage(s) observed that the secretion of pancreatic juice initiated by the introduction of HCl into the duodenum was not annulled by atropine. Similarly Bayliss and Starling observed that atropine had no effect on the amount of pancreatic juice produced under

the influence of secretin. During the course of these experiments, I have observed that atropine appears to augment the amount of juice secreted under the influence of secretin. Thus after the cessation of a flow of pancreatic juice initiated by the intravenous injection of secretin, the flow recommences after the injection of 5 mgrm. of atropine. Atropine therefore appears to stimulate the secretory activities of the pancreas. This anomalous action, however, cannot be produced in an animal which has not previously been treated with secretin. The effect is probably due to the paralysis by atropine of the vagal endings contained in the plain muscle fibres which lie along the pancreatic ducts, as described by Anrep(9). The relaxed muscle thereby allows the juice previously held up in the constricted ducts to issue from the gland. Although atropine has no influence on the volume of pancreatic juice secreted under the secretin stimulus other than that just described, yet it has a marked effect on the composition of pancreatic juice. Thus in one experiment pancreatic juice, obtained from a cat by the intravenous injection of secretin was collected in six portions of 3 c.c. After the second sample was collected 10 mgrm. of atropine sulphate was injected into the blood. The following figures show the quantities of  $\text{NaHCO}_3$ , trypsinogen, amylase and lipase contained in the six portions of juice:

	Before atropine		After atropine			
$\text{NaHCO}_3 N$	.15	.14	.146	.134	.132	.126
Trypsinogen	2800	2400	1100	900	650	600
Amylase	500	340	140	60	70	50
Lipase	51	32	5	2	1	1

After the atropine injection the rate of secretion and the quantity of bicarbonate in the juice was practically unaltered, but there was a considerable diminution in trypsinogen, amylase and lipase. This fact becomes more evident if the average composition of 6 c.c. of juice secreted before and after the intravenous injection of atropine is considered thus:

	6 c.c. juice before atropine	6 c.c. juice after atropine
$\text{NaHCO}_3 N$	.145	.140
Trypsinogen	2600	1000
Amylase	420	100
Lipase	42	4

The figures afford confirmatory evidence in favour of the hypothesis that secretin causes a flow of bicarbonate solution through the cells of the pancreas whilst the vagus nerve determines the elaboration of enzymes in the cells of the gland.

*The composition of pancreatic juice before and after cutting the vagus nerves.* As a corollary to the above experiment the effect of vagal section on the volume and composition of pancreatic juice secreted under the influence of secretin was determined. After the secretion of two portions of pancreatic juice, each containing 3 c.c., both vagi were cut in the neck. After this procedure three additional quantities of 3 c.c. were collected. The analysis of these successive portions of juice gave the following figures:

	Before vagal section		After vagal section		
NaHCO <sub>3</sub> N	1.134	.138	.138	.132	.128
Trypsinogen	1250	800	330	280	100
Amylase	520	200	75	75	50
Lipase	30	19	9	2	5

The results are comparable to those observed before and after the intravenous injection of atropine. The rate of secretion and the quantity of alkali (0.136 N. NaHCO<sub>3</sub>) contained in the juice was unaltered by section of the vagi. The enzyme content, however, was considerably diminished, trypsinogen falling from 1000 to 300 units, amylase from 360 to 75 units, and lipase from 25 to 5 units. This result indicates that the enzyme content of the juice is determined by impulses along the vagus nerves probably reflexly through the vagus nuclei in the bulb.

#### DISCUSSION OF RESULTS.

The question arises as to the importance of secretin in the mechanism for pancreatic digestion. The experiments recorded indicate that secretin stimulates the cells of the pancreas to produce a copious flow of a dilute solution of sodium bicarbonate which carries the pancreatic enzymes with it. The metabolism of the enzymes of the pancreas, however, appears to be under the control of the vagus nerves, and in this respect the results confirm the previous conclusions of Babkin and Savitsch. Secretin therefore ensures the presence in the intestine of an adequate supply of sodium bicarbonate to preserve the neutrality of the intestinal contents during the process of digestion. The importance of the secretion of this alkaline fluid is evident from the fact that its reaction is such as to secure an optimal medium for the activity of lipase, amylase and trypsin.

There are a large number of well established observations that normal intestinal digestion may be associated with the complete absence of hydrochloric acid from gastric juice. In these cases, on the hypothesis of Bayliss and Starling that secretin formation depends on the action

of hydrochloric acid ou prosecretin contained in the duodenal mucosa, no secretin action is possible. Therefore the vagal control of pancreatic secretion may play the dominant role in pancreatic digestion as stated by the Russian School of Physiologists, whereas the production by secretin of a copious flow of a dilute bicarbonate solution in which the pancreatic enzymes are contained may not be essential to normal digestion.

#### SUMMARY.

1. Pancreatic juice secreted after the intravenous injection of secretin contains a constant quantity of  $\text{NaHCO}_3$  (approximately 0.14 *N*) but diminishing quantities of trypsinogen, amylase and lipase as secretion proceeds.

2. The diminution in the quantities of enzymes secreted is not due to the exhaustion of the gland, since after long continued secretion the quantities of enzymes may be increased to their original values by vagal stimulation.

3. Removal of vagal control from the cells of the pancreas either by atropine or by section of the vagi in the neck diminishes the quantities of enzymes contained in pancreatic juice, but does not diminish the quantity of bicarbonate contained in this juice, nor the rate of secretion.

4. The hypothesis is put forward that the enzyme content of pancreatic juice is determined by the vagus nerves, whereas the quantity of bicarbonate solution in which these enzymes are contained is determined by secretin. On this hypothesis, secretin may play a subsidiary part in pancreatic digestion, since it only ensures the presence in the intestine of an optimal reaction for the activity of the pancreatic enzymes.

The expenses of this work were defrayed by a grant from the Government Grant Committee of the Royal Society

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# INSULIN AND THE PITUITRIN "FAT LIVER."

By R. COOPE.

*(From the Department of Biochemistry, University of Liverpool.)*

IN a recent paper, Coope and Chamberlain (1925)(1) showed that subcutaneous injections of pituitrin into rabbits resulted in fatty infiltration of the liver. Hitherto such infiltrations have usually been associated either with severe lesions of the liver cells, as in phosphorus and delayed chloroform poisoning, or with the persistent glycosuria of diabetes and phloridzin intoxication. The pituitrin effect is not accompanied by damage to the liver cell, nor do the injections cause hyperglycæmia or glycosuria.

It is a well-known clinical fact that insulin can prevent diabetic lipæmia and fatty infiltration of the liver; and Campbell and Macleod (1924)(2) have shown that in depancreatized dogs also the effect on lipæmia is very rapidly developed ("within a few hours"), although the effect on the liver-fat is not so prompt.

This paper records experiments made to obtain more light on the interpretation of these facts, by investigating whether insulin modifies the action of pituitrin in producing fatty livers. As in the previous work, rabbits of various body and liver weights were used, with similar feeding and general management. Each separate batch of pituitrin used (Parke Davies and Co.) was tested to ensure that it was effective in producing a fatty liver. In each experiment with pituitrin plus insulin, 4 c.c. of pituitrin in gum solution were injected into the right flank of the animal, and at the same time 40 to 45 units of insulin (B.D.H.) in 6 c.c. of gum solution were injected into the opposite flank. The animals were allowed their usual food during the experimental period; if this happened to involve the night, they had little or no opportunity of feeding before they were killed early the following morning. Feeding or abstinence, however, made no obvious difference to the results. The animals were killed after times varying from 4 to 24 hours, and the fatty acid of the liver was estimated as in the previous work. They appeared to suffer no ill effects from the injections, and there were no symptoms of hypoglycæmia.

The results of the experiments with pituitrin plus insulin are given in Table I. In certain cases, paired experiments were made, in one of which pituitrin was given alone—these latter are given in the table for comparison.

Fig. 1 facilitates comparison between all the pituitrin experiments (with gum) of this and the previous paper, and those in which pituitrin plus insulin were given simultaneously.

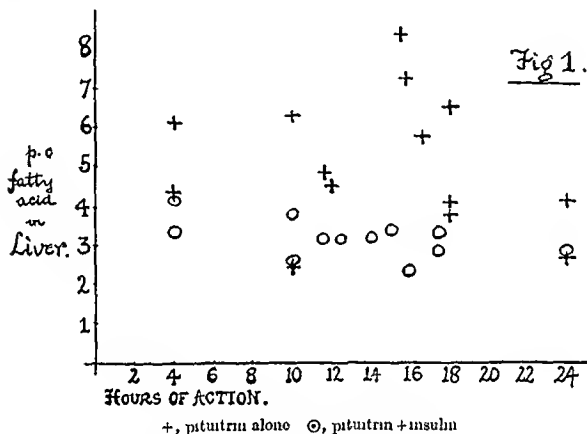


TABLE I. Percentage of fatty acid in liver.

A. 4 c c. pituitrin with gum, and 40 units of insulin with gum, except as mentioned			B. Paired experiments with pituitrin alone (4 c c. with gum)		
Exp.	p c.	Hours of action	p c.		Exp
1*	2.29	10	—		—
2	2.56	10	2.52		13
3†	2.90	17½	4.11		15
4	2.06	24	2.85		14
5*	3.11	12½	—		—
6	3.16	11½	4.84		17
7*	3.19	14	—		—
8	3.35	17½	See Exp 15		
9*	3.43	4			
10	3.46	15	4.25		16
11	3.88	10	6.25		19
12*	4.21	4	6.11		18
Mean 3.21			Mean 4.42†		

\* 15 units.

† 30 units of insulin

‡ Mean of exps. in previous paper (Coope and Chamberlain) 5.34

It is of interest to note (Exps. 16 and 18) that the pituitrin effect on the fat-content of the liver was present even after so short a time as 4 hours after the injection—a period which had not been explored in the previous work.

From the experiments, it is clear that a single dose of insulin given simultaneously with pituitrin profoundly modifies, and in many cases completely prevents, the pituitrin effect on the liver-fat. We have in this fact evidence of an antagonism, probably of significance in the normal animal, between the effects of pituitrin and insulin, analogous to that observed by Burn(3) for the blood sugar.

I am indebted to the Medical Research Council for a grant covering part of the expenses of the research.

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# THE MAXIMUM WORK OF THE SEVERAL MUSCLES OF THE FOREARM. BY J. H. O. REIJS.

SOME time ago I devised a dynamometer(1) for determining the maximum amount of work performed in certain voluntary movements in man, and have published(2) the results obtained in plantar flexion of the foot, abduction and adduction of the leg, flexion of the forearm and of other movements. I give here some observations on the relative parts played by the several muscles in flexion of the forearm. These start from the curve I have already given(2) which is obtained when the ordinates are the work done and the abscissæ the angle of flexion of the elbow. The curve is reproduced in *R*, Fig. 2. The curve is practically the same (except for level) as that obtained by Bethe and Franke(3) using an entirely different dynamometer so that the curve in its general features may fairly be taken as accurate. With the help of this curve it is

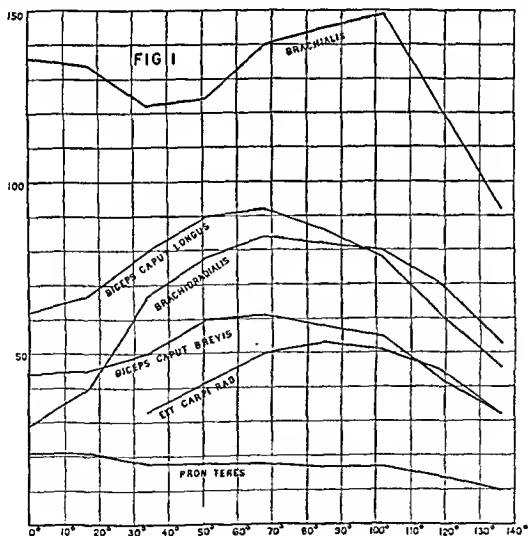


Fig. 1.

possible to calculate from the data given by Braune and Fischer(4) the share taken by each flexor muscle at every moment of the movement. When we do this and present the results graphically, Fig. 1 is obtained.

Braune and Fischer have replaced the flexors of the forearm appliances by cords running between pins, which were attached to the bone at the connecting-points and by a small hook fixed in the centre of the plane of origin of these muscles. From the shortenings which the cords showed when bending the elbow joint, the rotation moments of the muscles were calculated. The authors calculated these for three possibilities: (a) that the muscular force remains constant during the movement; (b) that the force decreases in direct proportion to the shortening; (c) that the force decreases as the square of the

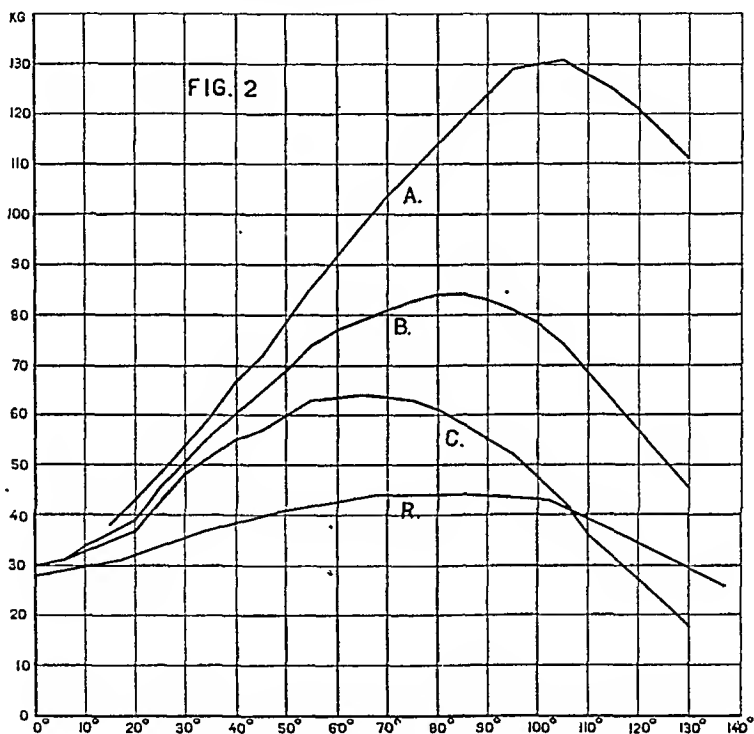


Fig. 2. Abscissæ=angle of flexion of the elbow.

shortening. In Fig. 2 I give curves representing these three cases and also (R) the curve I obtain with my dynamometer. The supposition upon which curve B is constructed is that which on our present knowledge is most probable. It will be seen that as regards its maximum, my curve has a general resemblance to curve B, but it does differ very considerably, and especially in its much greater flatness. Since the actual force employed in flexion is I hold represented by my curve I conclude that it is inadmissible to calculate from Braune and Fischer's curves the power with which the movement will take place in the body.

The most remarkable point in Fig. 1 is the irregular line of the m. brachialis internus. But this is exactly the muscle, which, owing to

its large and irregular plane of origin, will give rise to inaccuracies if instead of its total origin a single point only is taken. In order to estimate the importance of this complication I have repeated the measurements of Braune and Fischer for the *m. brachialis internus*, but I took for the muscle not a single thread, but four threads which were fastened at one point, the tuberculum ulnæ, and originated from four places namely: (1) the centre of the plane of origin, (2) the lower point of this plane, (3) and (4) the two proximal, medial and lateral points of the plane, which is situated around the connecting place of the *m. deltoideus* in the form of a horseshoe. A graphical representation of these measurements is given in Fig. 3.

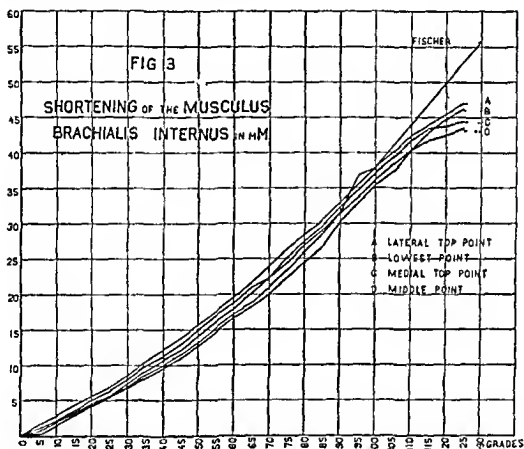


Fig. 3. A, lateral proximal point, B, distal point; C, medial proximal point; D, middle point.

To my astonishment my four curves are not only practically equal, but they are also identical with the curves of Braune and Fischer. So it seems to be irrelevant which point of the plane of origin is taken.

It is possible to calculate from my experiments, which give values for the power with which the movement occurs, and from the proportion numbers of Braune and Fischer, the power per square centimetre of cross section of the muscles. The maximum force will be at the greatest length of the muscles, *i.e.* as regards the flexors of the arm,

when the arm is extended. As, however, in this position the m. flexor carpi radialis has a stretching action in three arms, I have left this muscle out of consideration. From Fig. 1 the values of the flexion components of these muscles at the beginning of the movement may be seen, namely:

Pronator teres	2.1 kg.
Brachialis internus	13.6
Biceps, caput longum	6.2
Biceps, caput brevis	4.4
Brachio radialis	2.9

The value  $\frac{dV}{da}$  from which the other component can be found is given by Braune and Fischer(4) for these muscles, when the arm is extended (average calculation) as:

Pronator teres	0.086
Brachialis internus	0.191
Biceps, caput longum	0.2005
Biceps, caput brevis	0.212
Brachio radialis	0.17

When we calculate from this the value of the other component and of the total muscular force, we get the following table, in which in the third column the cross section, from Braune and Fischer is given, and in the fourth column the power per square cm.:

	Component kg.	Total power kg.	Cross section cm. <sup>2</sup>	Power per cm. <sup>2</sup> kg.
Pronator teres	24.4	24.5	4.2	5.83
Brachialis internus	71.2	72.4	12.4	5.83
Biceps, caput longum	30.9	31.6	5.4	5.85
Biceps, caput brevis	20.7	21.1	3.6	5.86
Brachio radialis	17.06	17.3	3.0	5.76

Average 5.82

The average of 5.82 agrees very well with my previous result 5.64 kg. for the foot muscles(5). Without attaching too much value to this number, I think it tends to confirm the correctness of my experiments on the absolute muscular force, and on the change of power during contraction and also the correctness of the operations which I have applied to the figures of Braune and Fischer. Further the very striking conformity between the values of the absolute muscular force for the flexors individually afford evidence of the validity of the calculations.

The maximum work of muscle has been estimated by A. V. Hill(6) and by Hansen and Lindhard(7) by a different method. As I have not made experiments by these methods I am unable to say what the cause of the difference is, but I think my method represents more accurately the conditions occurring in the body.

## SUMMARY.

1. With the use of a special form of dynamometer, a curve of the force excited at each moment of flexion of the forearm of man has been obtained.

2. Combining the values so obtained with the data for the rotation movements of the muscles given by Braune and Fischer, the share taken by each muscle at each moment of the movement and the absolute power per square centimetre of cross section of each muscle have been calculated.

3. The absolute power of each muscle so calculated is approximately the same, the average of all being 5.82 kilograms.

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# ON THE COMBINATION OF NITRIC OXIDE WITH HÆMOGLOBIN. BY M. L. ANSON AND A. E. MIRSKY<sup>1</sup>.

*(From the Physiological Laboratory, Cambridge.)*

IN the course of some investigations on the relative affinities of oxygen and carbon monoxide for hæmoglobin it was desired to have a third gas that combines loosely with hæmoglobin. It has long been known that nitric oxide reacts with hæmoglobin and that it can displace carbon monoxide from its combination with hæmoglobin. It has been supposed that the reaction between NO and hæmoglobin is of the same nature as that between CO and hæmoglobin; that NO displaces CO from its combination with hæmoglobin for the same reason that CO displaces O<sub>2</sub>.

We found that in the reaction between NO and HbO<sub>2</sub> or HbCO the first step is the formation of methæmoglobin. (The anomalous results with reduced hæmoglobin will not be considered here.) A solution of HbO<sub>2</sub> or HbCO is injected by means of a syringe into a tonometer containing NO diluted with hydrogen. The events are followed spectroscopically. If the solution is kept alkaline, alkaline methæmoglobin first appears, and this then reacts with NO to form what is ordinarily known as NOHb, but which should be called NOMHb because it is with methæmoglobin that NO combines. Previous workers have always kept the solution alkaline to prevent it from becoming too acid as a result of possible nitrous acid formation. Not wishing the solution to become too acid they never had the opportunity of observing what occurs in even faintly acid solution. If, however, the experiment is repeated in slightly acid medium, acid methæmoglobin first appears and NO reacts with this to form a new compound whose absorption spectrum and colour are different from those of alkaline NOMHb. The new form is much browner than the alkaline form and its two-banded spectrum is more distinct. The  $\alpha$  band is at 5687 Å. In a few minutes, however, the new form goes over into the alkaline form of NOMHb. If this solution is now made still more acid, this transformation is reversed and the new form reappears. In a solution that is acid enough to begin with, the alkaline form never appears at all. After finding out that the first step

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in the reaction is the formation of methæmoglobin we always began the experiments with methæmoglobin itself.

Methæmoglobin is an indicator (1, 2). At  $pH$  9 methæmoglobin exists entirely in the alkaline form with its characteristic colour and spectrum; at  $pH$  8 it is entirely in the acid form, the colour and spectrum of which are entirely different from those of the alkaline form, and at intermediate  $H$ -ion concentrations there are various mixtures of these two forms. If the  $pH$  is plotted against the percentage composition of these mixtures as determined spectroscopically, the curve obtained is the characteristic curve of the influence of  $pH$  on the dissociation of a weak acid. Alkaline methæmoglobin, then, is simply the ionised and acid methæmoglobin, the un-ionised form of a weak acid whose dissociation constant (actually about  $10^{-8.5}$ ) is given by the turning point of methæmoglobin as an indicator.

NOMHb also proves to be an indicator with a turning point at about  $pH$  5.6. In other words NOMHb is of the order of a thousand times as strong an acid as is methæmoglobin. This explains why at certain  $H$ -ion concentrations although acid NOMHb is formed it changes over into the alkaline form. The NO combines with un-ionised methæmoglobin to give un-ionised NOMHb which at that  $pH$  proceeds to dissociate. If the acid NOMHb is to be permanent, the solution must be much more acid than is necessary merely to have methæmoglobin in the un-ionised form; it must be acid enough to assure that the NOMHb formed will remain completely un-ionised.

The hydrogen-ion concentration influences not only the spectrum of methæmoglobin but also its affinity for NO. The NO of alkaline NOMHb cannot be removed with the pump. The NO of acid NOMHb, on the contrary, can be pumped off easily. The  $[H]$  is known to have a similar influence on the affinity of hæmoglobin for oxygen, and this effect has been interpreted as meaning that oxyhæmoglobin is a stronger acid than is reduced hæmoglobin. The same interpretation applies to the analogous NOMHb case. The relations between the equilibrium and the dissociation constants are given by the mass law equations which apply generally to dilute solutions.

$$(1) \frac{[H^+][MHb^-]}{[HMHb]} = K_1.$$

$$(2) \frac{[H^+][NOMHb^-]}{[NONMHb]} = K_2.$$

$$(3) \frac{[NO][MHb^-]}{[NOMHb]} = C_1.$$

$$(4) \frac{[NO][HMHb]}{[NONMHb]} = C_2.$$

$$\frac{(1)}{(2)} = \frac{(3)}{(4)} \text{ or } (5) \frac{K_1}{K_2} = \frac{C_1}{C_2}.$$

The exact affinities of acid and alkaline methæmoglobin for NO have not been measured, but their approximate values can be estimated by comparison with the known affinities of hæmoglobin for O<sub>2</sub> and CO. Alkaline methæmoglobin has a much *greater* affinity for NO than Hb has for CO and acid methæmoglobin has much *less* affinity for NO than hæmoglobin has for O<sub>2</sub>. Since hæmoglobin has about four hundred times greater affinity for CO than for O<sub>2</sub>,  $\frac{K_1}{K_2}$  must be of the order of a thousand. It follows from equation (5) that NOMHb is about a thousand times as strong an acid as is methæmoglobin. This value agrees with the estimate already made *independently* from the spectroscopic data.

In contrast, oxyhæmoglobin is only 25 times(3) as strong an acid as reduced hæmoglobin. In certain hæmocyanins, however, the difference between the dissociation constants of the oxygenated and reduced forms appears to be of the same magnitude as the difference between the dissociation constants of NOMHb and MHb. In these hæmocyanins (at body temperatures) the oxygen can be set free only if the solution is made acid, say by bubbling CO<sub>2</sub> through it(4).

#### SUMMARY.

1. Nitric oxide forms a loose combination with methæmoglobin.
2. Nitric oxide methæmoglobin is about a thousand times as strong an acid as is methæmoglobin.

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## A CLOSED CIRCUIT HEART LUNG PREPARATION.

### I. Effect of Alterations in Blood Volume.

By I. DE BURGH DALY.

(From the Physiology Institute, Cardiff.)

IN experiments, which were carried out to investigate the effect of changes in the capacity and peripheral resistance of the vascular system upon the arterial and venous blood-pressures, Knowlton and Starling's heart lung preparation(1) was converted to a closed circuit system. Their preparation I shall refer to as the open circuit system. Dogs were used in all experiments and were anaesthetised with chloralose injected intravenously 1 decigram per kilo body weight.

The arrangement of the apparatus used is depicted in Fig. 1. The parts lettered A, A', B, C, D, E, F, G, H, I represent the open circuit

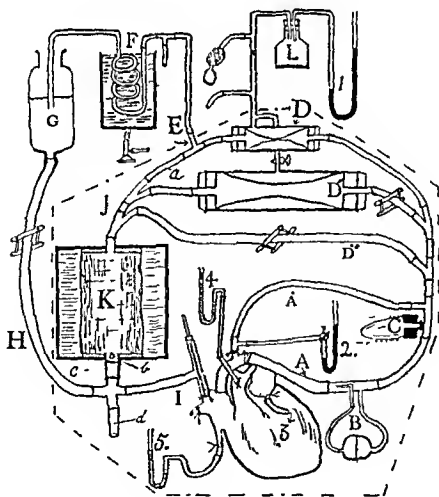


Fig. 1

system described by Knowlton and Starling, the only departure from the usual form being the insertion of a stout rubber finger stall (*C*) on the arterial side in place of the air cushion. The finger stall had a capacity of 25 c.c. at atmospheric pressure and its increase in volume at different arterial pressures is shown in Fig. 2.

The closed circuit system *A*, *A'*, *B*, *C*, *D*, *E*, *J*, *K*, *I* was set up so that all the component parts were at heart level. *K* was a length of motor cycle inner tube, 20 cm. in length and 250 c.c. in capacity, it served in place of the venous reservoir. The water in the thermostat surrounding *K* was at such a level that the tube did not completely collapse when open to the air. A pressure volume curve of *K* is shown in Fig. 2.

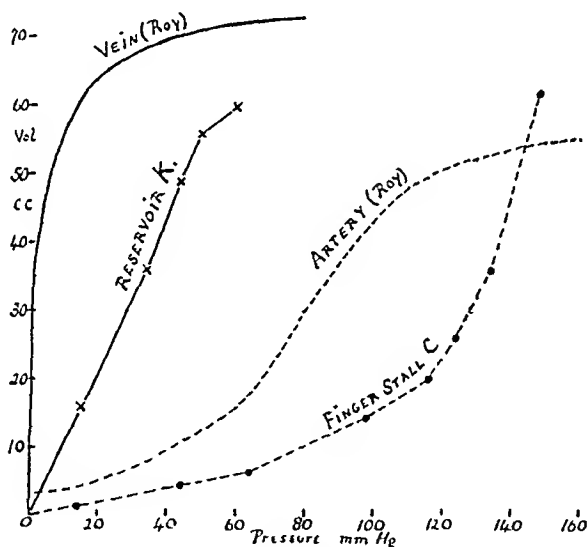


Fig. 2.

The artificial resistance (*D*) was shunted by a larger resistance of similar type of 200 c.c. capacity (*D'*) and also by a plain rubber tube (*D''*). All connections on the venous side were made with rubber tubes 13 mm. in bore. The total capacity of the artificial circulation less the heart and lungs was approximately 325 c.c. In later experiments additional temperature control was effected by inserting a glass tube spiral at *A'* which was kept in a thermostat.

*Methods of recording.* Starling's heart lung preparation was first made so that the blood flowed by way of *A*, *A'*, *B*, *C*, *D*, *E*, *F*, *G*, *H* and *I*, all other channels leading from this circuit being clamped off. Manometers were then inserted for recording pressures. The venous pressures

were taken by the introduction of cannulae into the right auricle through the inferior vena cava and into the tip of the left auricular appendage, these were connected by rubber tubing to two burettes half filled with 0.9 p.c. saline (s.g. 1.015). The open ends of the burettes communicated with piston recorders. Centimetre scales placed behind the burettes allowed of direct readings of the venous pressures, these were taken as controls as often as possible. The arterial blood-pressure was recorded with a mercury or Hurtle manometer connected to the cannula in the brachiocephalic artery.

A straight piece of glass tubing, 3 mm. in bore and with a small rim at one end, was inserted into the pulmonary artery secured with a purse string suture so that its projection into the lumen of the artery was only equal to the thickness of the rim. The cannula was firmly fixed at right angles to the long axis of the artery and connected to a membrane manometer. The output of the ventricles was recorded by means of a Henderson's cardiometer connected either to a water volume recorder or to a 50 c.c. piston recorder. In some experiments Pitot tubes were placed in the channel between the aorta and peripheral resistance (Fig. 1 B), the pressures being recorded with a Hurtle(?) differential manometer. The results were in the main the same. It was not possible to measure the cardiac output by withdrawing blood from the circulation since any alteration in the amount of blood circulating in a closed system of tubes will cause large variations in the arterial and venous pressures.

*The closed circuit system.* When all the manometers had been inserted and adjusted the reservoir *G* was filled three quarters full of blood. The clamp at "*a*" was then removed and blood allowed to flow into the reservoir *K*, the displaced air escaped through a vertically placed tube at "*b*". The blood was allowed to flow into *K* until it rose to a height of about 5 cm. in the tube "*b*" which was then closed. No difficulty was found in displacing all the air from the reservoir *K* and the adjacent tubing if suitable manipulations were employed, in general it was sufficient to fill the reservoir intermittently and let the blood flow onwards in jerks and at the same time to massage the reservoir towards the opening at "*b*".

The open circuit system was then switched over to the closed circuit system. The tube leading from the reservoir *G* was clipped at *H*, the tube carrying blood to the thermostat *F* clipped at *L*, and the clamp at *C* removed. If these three operations were carried out in quick succession it was found that the venous pressures remained approximately

the same. If there was too much blood in the reservoir *K* and the venous pressures rose, blood was withdrawn through the side tube "*d*"; too small an amount of blood in the reservoir was compensated for by running in blood from the reservoir *G*. The path of the blood through the closed system was then by *A*, *A'*, *B*, *C*, *D*, *E*, *J*, *K* and *I*. Any leakage of blood from the system was detected by the gradual fall in venous pressures and was compensated for by the injection of blood, an operation which had to be performed every ten to twenty minutes. The injection was carried out with the aid of a graduated pressure bottle placed in the thermostat surrounding *K* the outlet of the bottle being connected to the tube "*b*." At the end of the experiments the zero of the venous pressures was registered at the level of the left auricular appendage.

In this preparation the effects of gravity flow on the right side of the heart are eliminated and the inflow to this side is determined by the *vis a tergo* maintaining the venous pressure. Since the right auricle can only receive as much blood as the left ventricle puts out, it follows that any increase in coronary flow will be at the expense of the peripheral flow. The pressure volume changes of the arterial and venous connections are given in detail, because any alteration in their values will cause corresponding changes in the pressure variations throughout the system. The curve of the finger stall *C* (Fig. 2) is the same shape as the curve for a contracted artery given by McWilliam(3), but differs from the artery curve constructed from data given by Roy(4) (Fig. 2).

*Results.* Five experiments were made; the protocol of one is given in Table I and the essential data of three others in Fig. 3.

TABLE I. Dog. 10 kg. Heart, 125 gm. Chloralose.

Pressure in left auricle mm. 0.9 p.c. NaCl	Pressure in I.V.C. mm. 0.9 p.c. NaCl	Blood- pressure	Heart rate	Output per beat in c.c.	Temp.	
72	54	58	120	4.1	36.0°	20 c.c. blood injected
92	69	68		6.0		10 " " "
107	77	78		8.4		10 " " "
115	82	85		9.2		10 " " "
120	87	90		9.6		10 " " "
130	90	96		9.8	35.8	10 " " "
142	100	100	120	10.0		10 " " "
160	112	104		10.4		10 " " "
205	139	106		11.6	36.0	20 " " "
155	102	104		11.0		20 c.c. blood withdrawn
125	82	92		9.2		20 " " "
105	72	74	114	8.4		20 " " "
80	65	56	108	7.3		20 " " "
68	60	52	112	6.4	36.0	20 " " "

The results demonstrate that an increase in the volume of circulating blood has, with the exception of minor differences, the same

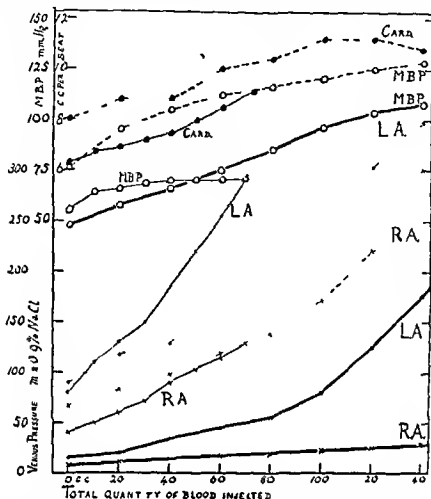


Fig 3 Influence of volume of circulating blood as —●, cardiometer output in c.c., ○, mean arterial pressure, , left auricular pressure, ×, right auricular pressure Exp 1, thin line Exp 2, broken line Exp 3, thick line

effect as an increase in venous inflow in the open circuit system. With outputs varying from 3.3 to 11 c.c. per beat, the pressure in the right auricle is lower than the pressure in the left auricle. The majority of experiments were performed with an initial output of approximately 500 c.c. per minute as measured by the cardiometer, at this output the pressure in the left auricle is slightly higher and the pressure in the right auricle slightly lower than the corresponding values given by Patterson and Starling<sup>(5)</sup>. Yas Kuno<sup>(6)</sup>, working with Starling's heart lung preparation with the pericardium removed found that with increasing venous inflow the rate of rise of pressure in the right auricle was greater than that in the left auricle. With intact pericardium, he found that the rate of rise of pressure in both auricles was approximately equal. In our experiments the pericardium was always open, but with increasing amount of circulating blood and therefore increasing cardiac output, the rate of rise of pressure in the left auricle was greater than

in the right auricle. These results were obtained with a constant peripheral resistance and suggest that as the blood flow increases either the functional capacity of the left ventricle becomes relatively less than that of the right ventricle or the amount of work performed by the left ventricle becomes relatively greater.

#### SUMMARY.

A closed circuit Starling's heart lung preparation is described.

An increase in the volume of circulating blood produces, with the exception of minor differences, the same effect as an increase of venous inflow in the open circuit heart lung preparation.

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# THE EFFECT ON MUSCLE CONTRACTION OF SYMPATHETIC STIMULATION AND OF VARIOUS MODIFICATIONS OF CONDITIONS.

BY DR HELENE WASTL (VIENNA).

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THE question, whether the sympathetic system has any direct influence upon striated muscle, has been widely discussed within the past ten years. Most observers, who have supported the theory that such influence exists, have considered that it consists of an increase of some form of tonic contraction. One of the difficulties in the way of this conception is, that no one has been able to cause increase of muscle tone by stimulating the peripheral end of any sympathetic nerve. Recently a different view of the mode of action of the sympathetic on striated muscle has been put forward by Orbeli. Orbeli and his pupil Ginezinsky<sup>(1)</sup> have described in a preliminary paper experiments on survival frog's muscle, which they consider show, that if the muscle is fatigued, its activity is more or less restored by stimulating the sympathetic. On Orbeli's view the sympathetic has all the effects on striated muscle that it has on the heart: increasing the height, strength and rapidity of the contraction and the conductivity of the muscle. On this view the action of the sympathetic might be regarded as primarily trophic and by its trophic influence secondarily increasing muscle tone. The action corresponds fairly closely to that which a number of observers have found to be produced on fatigued muscle by adrenaline, and Gruber<sup>(2)</sup> has suggested that adrenaline has some kind of trophic action on the muscle either direct or by aiding in the removal of metabolic products<sup>3</sup>. Clearly, if similar results to those obtained by Ginezinsky in frog's muscle were obtained on mammalian muscle (and his results in frog's muscle were confirmed) the doubts as to the existence of any relation of sympathetic nerves to skeletal muscle would hardly be tenable.

<sup>1</sup> In a letter to Prof. Langley, Prof. Orbeli mentions a number of confirmatory experiments made in different ways by others of his pupils.

<sup>2</sup> According to L. Lapique the chronaxie of the fatigued muscle, which is very much increased, can be brought back to its normal value by treating the fatigued muscle with adrenaline.

From this point of view I have, at Prof. Langley's suggestion, investigated the question first in mammalian and then in frog's muscle.

*Sympathetic nerve stimulation during fatigue of the  
tibialis anticus muscle of the cat.*

Ginezinsky's experiments were made on the posterior part of a frog severed from the anterior part. The contraction of the gastrocnemius was recorded; the 8th and 9th spinal nerve roots stimulated to produce contraction of the muscle and the sympathetic trunk stimulated at the level of the 7th ganglion during fatigue of the muscle. The experiments were of several types (isotonic and isometric contractions, fatigue by single shocks and by a short series of tetani) but as the same conclusion was drawn from each of these, one type is sufficient to test the results. I have taken that first mentioned. In this, the roots were stimulated with simple induction shocks and when the muscle was strongly fatigued and in contracture, the sympathetic was stimulated. Ginezinsky found that after a long latent period the contractions gradually increased in height, reached their maximum a short time after the end of the sympathetic stimulation and very slowly lessened.

My experiments were made in cats anæsthetised by subcutaneous injections of urethane and subsequently with chloroform-ether. A tracing was taken of the contractions of the tibialis anticus muscle, the proximal origin of the muscle being fixed by clamping a rod passed through the knee-joint. The anterior roots of the 6th and 7th lumbar nerves (or one of these nerves) were stimulated with single break shocks produced by an interrupter driven by an electric motor. The make shocks were short circuited and the number of breaks administered to the roots were varied by a resistance.

Usually 100-220 stimuli per minute were used, a 1.8 to 2 volt cell being in the primary circuit. The sympathetic chain of the same side was reached retro-peritoneally through a cut parallel with the vertebral column a little anterior to the edges of the processi transversi and cut just below the 5th lumbar ganglion, this being the last in the cat which (usually) receives a white ramus. The sympathetic was stimulated between the 5th and 6th ganglia with faradic currents from a second coil. Only those experiments are counted, in which sympathetic stimulation caused a prompt erection of the hairs of the tail, thus indicating a good excitability of the nerve. In some cases the blood-pressure was registered from the carotid artery.

The general course of these contraction curves, especially of the



tibialis anticus of the cat (which has always been a favourite in studies on fatigue) with their returning features of the staircase phenomenon, of the initial more or less great decrease of the height of contractions, of the subsequent continuation of the contractions for a long time on a level height ("fatigue level" or "steady state"), and, finally, of the more or less steep decline in height till exhaustion, are too well known to render it necessary to describe and picture them anew.

The sympathetic nerve was stimulated with various strengths of faradic currents (coil distance about 30 to 5 cm.) during various periods (about 30" to 2 mins.) at the different points of these contraction curves: during the initial decline, or when the muscle was working on the fatigue level, or when the muscle was already more or less fatigued, or when it responded with feeble contractions only.

In the greater number (six cats) of successful sympathetic experiments (ten cats) *no effect* upon the muscle curve was obtained by sympathetic stimulation.



Fig. 1. Uppermost tracing: contractions of anterior tibial muscle of cat at fatigue level; (1) the anterior roots of 6th and 7th lumbar nerves stimulated 220 times a minute; 2nd record: time (as in the other figures), in 10 second intervals, 3rd record: stimulation of sympathetic (coil distance 15 cm.) Lowest tracing: signal of the anterior root stimulation (omitted in the other figures).

Fig. 1 is an example of the complete absence of effect of stimulating the sympathetic during contractions of the musc. tibialis anticus of the cat at the fatigue level. The contractions continued at the height shown for nearly one hour, during which the sympathetic was stimulated a number of times with different strengths of current, all strong enough to cause good erection of the hairs of the tail.

In three animals, sympathetic stimulation caused a marked decrease in the height of the contractions; the decrease was gradual and only gradually passed off after cessation of the sympathetic stimulation. The extent of the decrease in any one experiment depended within certain limits upon the strength of stimulation of the sympathetic. In

Fig. 2 (a) and (b) are taken from one experiment and (c) from another.

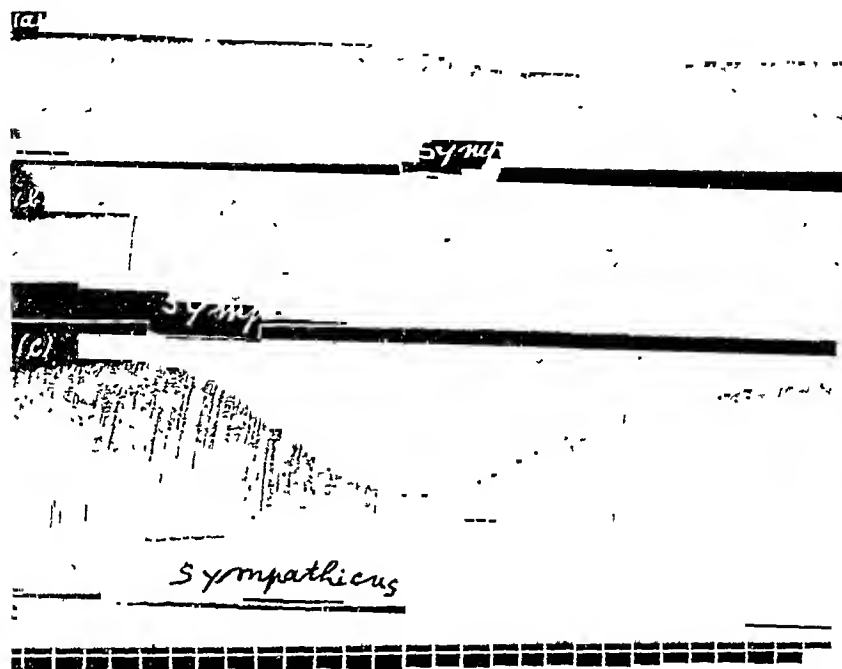


Fig. 2. (a) Moderate (22 cm.); (b) stronger (20 cm.) stimulation of sympathetic during fatigue level of anterior tibial muscle (stimulation of anterior root of 7th lumbar nerve 180 times per min.); (c) strong (16 cm.) stimulation of sympathetic from another experiment (anterior root of 6th lumbar nerve stimulated 160 times per min.).

In one experiment only there was a slight increase in height twice coincident with sympathetic stimulation, but in this experiment there were also some slight rises of the muscle curve independent of sympathetic stimulation, and the other sympathetic stimulations tried had no effect.

I conclude from these experiments that sympathetic stimulation has no direct effect on striated muscles undergoing marked fatigue in mammals (cats). It may have an indirect effect by decreasing the blood supply, but this causes a decrease and not an increase in the contractions of the fatigued muscle. A decrease in muscular efficiency was not found in all cases. But in a working muscle the vessels are kept strongly dilated by local chemical influences, so that vaso-constrictor impulses passing down the sympathetic may be unable to produce such a considerable contraction of vessels in the muscle as to influence its working power. That is probably the case when the blood-pressure is high and the

blood supply of the muscle abundant. Further, it is a common experience that the degree of vascular contraction in sympathetic stimulation varies with the conditions of the animal and the degree of anaesthesia.

As regards the action of adrenaline in fatigue, I have made a few experiments only. Observations on the tibialis anticus muscle of the cat have been made by Cannon and Nice(2), and by Gruber(3). Cannon and Nice obtained an increase in the working power of the muscle on stimulating the peripheral end of the splanchnic nerve. Part of this increase they considered was due to the action of adrenaline on the muscle. Gruber injected adrenaline and found that it increased the height of contraction and lowered the threshold of stimulation and shortened the duration of the latent and contraction period both in fatigued and in unfatigued muscle.

The conditions are complex, for a small dose of adrenaline causes some vascular dilatation in muscle (Hartmann and Fraser(4), Gruber and others) and whilst a large dose reduces the muscle circulation, there may be an intermediate stage in which the vaso-constriction produced in the visceral area and the consequent high blood-pressure may overcome the feeble vaso-constrictor action in the muscles. My experiments were made in the manner given above, adrenaline-chloride (Parke, Davis and Co.) being injected into the jugular vein instead of the sympathetic being stimulated. The blood-pressure of the carotid artery was recorded.

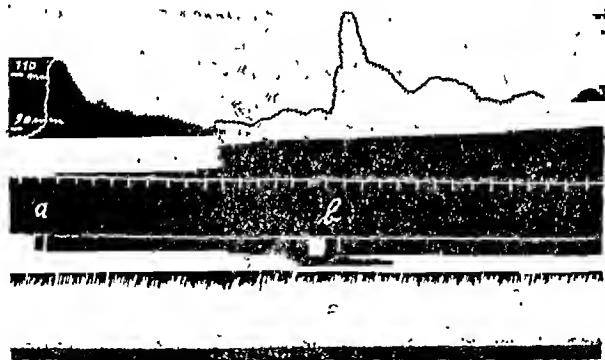


Fig. 3. Top tracing = carotid blood-pressure: (a) injection of .5 c.c. adrenaline .001 p.c. into jugular vein; (b) of 75 cm. .001 p.c. Lowest tracing: anterior tibial muscle, 7th lumbar anterior root (stimulated).

With small doses, which caused a fall of blood-pressure or with somewhat larger doses, which caused a brief moderate rise of blood-pressure, there was sometimes a slight gradual rise in the height of the contractions of the tibialis anticus muscle, but I did not obtain this constantly (cp. Fig. 3). On the other hand, in a number of experiments made, a somewhat larger dose of adrenaline sufficient to cause a large though not protracted rise of blood-pressure caused a decrease in the height of the contractions (Fig. 4), and this was not followed by an increase in height, *i.e.* any lessening in fatigue which the adrenaline may have caused was more than antagonised by the reduced blood supply.

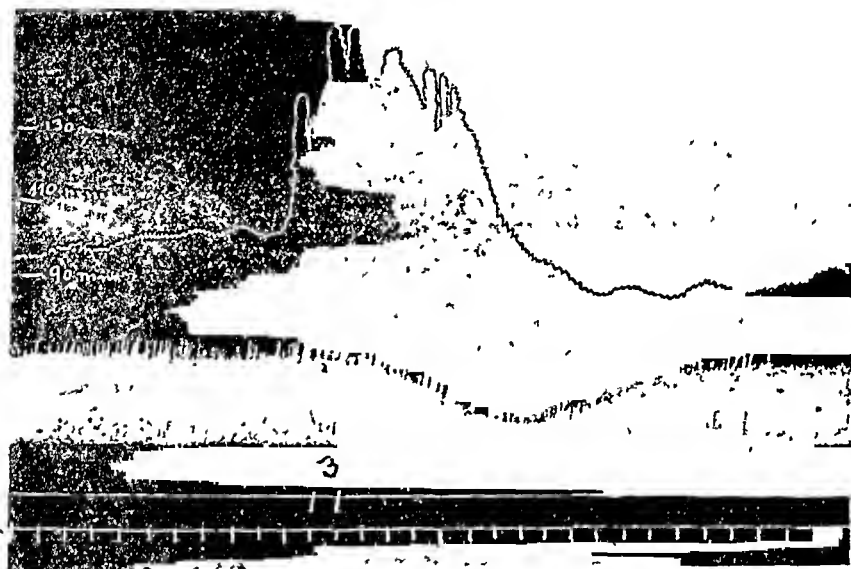


Fig. 4. Injection 1.25 c.c. 0.01 p.c. adrenaline (at 3).

#### *Effect of stimulation of the posterior roots.*

Since decreased blood supply of the muscle whether brought about by sympathetic stimulation or by injecting adrenaline causes decreased height of contraction in the fatigue curve, increased blood flow might be expected to increase the height of contraction.

An attempt was made to increase the blood supply of the muscle by stimulating the posterior roots of the 6th and 7th lumbar nerves. Few direct experiments have been made on antidromic vaso-dilation in muscle, but Bayliss(5) found that after removal of the skin of the hind limb a stimulation of posterior roots caused a slight increase in the volume of the limb. In the course of taking contraction curves of the

musculus tibialis anticus of cats, as described above, the posterior roots of the 6th and 7th lumbar nerves were stimulated either with slow stimuli (single induction shocks between 100-140 per minute) or with suitable faradic currents. No trace of effect upon the muscle curve was found either on non-fatigued strongly contracting muscles or in muscles in various states of fatigue. In order to meet the possible objection, that the sympathetic vaso constrictor fibres were in high tone and so prevented vaso dilator action the experiment was repeated after section of the abdominal sympathetic below the 5th lumbar ganglion, without any alteration however in the negative result.

It seemed possible that an effect might be obtained if an interval of inactivity were given to the muscle, during which period a stimulation of the posterior root might provide better conditions for the recovery of the muscle through antidromic vaso dilation. This was not found to be so (cp. Fig. 5)

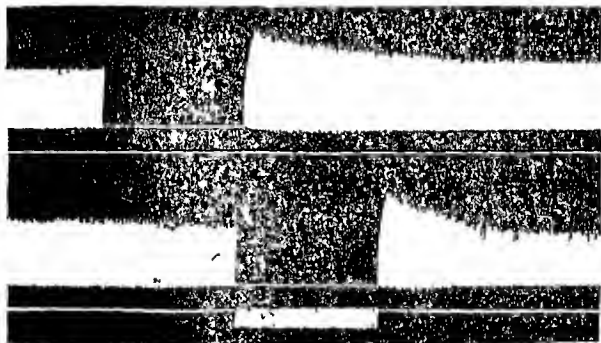


Fig. 5 Contractions of anterior tibial muscle produced by stimulating the anterior root of 7th lumbar nerve (120 times per min.) Interval 60. During the second interval the posterior roots of the 6th and 7th lumbar nerves were stimulated (100 times per min., make and break shocks). The lower curve follows on the upper

The negative results of these experiments might be due either to the absence of antidromic vaso dilation in the muscle or—as seems more probable—to the dilatation consequent on contraction of the muscle being already practically maximal, for it has been shown by Gaskell<sup>(6)</sup> and others that stimulation of a motor nerve to a voluntary muscle produces a very great vaso dilation therein.

*Sympathetic nerve stimulation during fatigue of the gastrocnemius of frogs.*

Since the mammalian experiment had given negative results it was obviously desirable—notwithstanding the apparent decisiveness of Ginezinsky's results—to try the effect of stimulating the sympathetic in the gastrocnemius muscle of the frog. The manner in which Ginezinsky's experiments were conducted, I have mentioned above. The method I employed was practically the same as that used by him in his isotonic series.

The experiments were carried out on large, mostly male frogs (*Rana temp.*); most of them were freshly caught (one experiment was done with a male American bull-frog, of 635 grm. weight, where the nerves are of course much longer). The frog was pithed, the left sympathetic chain was reached through a cut in the side of the animal without removing any viscera; the sympathetic was tied at the 5th ganglion and the sympathetic chain very carefully isolated to a little below the 7th ganglion, the intervening rami communicantes being cut, so that it was possible to stimulate the nerve at the region of the 7th ganglion. Then the spinal cord was exposed, the left 8th and 9th anterior roots (in some cases also the 7th root) were tied, usually separately, and the spinal cord with the rest of the roots were removed. In nearly all experiments the aorta was cut and there was no circulation in the hind limbs. In a few, however, the vessels were left intact and a very feeble circulation existed. This apparently made no difference to the results. The tendon of the left gastrocnemius was attached to an isotonic lever and the muscle allowed to pull against a load of 15–20 grm. according to its strength.

The roots were stimulated with a pair of small platinum electrodes partly with single induction shocks applied with various frequency (between 50–20 shocks per minute), partly with short faradic currents each of a duration of ca.  $\frac{3}{4}$ –1 second, at a frequency between 30–15 per minute. The sympathetic was stimulated for various lengths of time ( $\frac{1}{2}$ –3 minutes) and with various strengths of current. Care was taken to keep the nerves in good condition and not to touch the electrodes during each experiment.

The roots were stimulated in the various experiments either each alone (in some cases also the 7th anterior root was found to be effective), or together. The sympathetic was stimulated with a suitable strength at almost every possible point of the fatigue curve of

the muscle in the different experiments, at the beginning when the muscle contracted strongly and regularly, at the first signs of developing fatigue, during the various degrees of fatigue and especially at the end of the fatigue curve, when the muscle was already nearly exhausted. Particular stress was laid upon the effects of the first stimulation of the sympathetic in each experiment, since in the absence of circulation there is probably a more or less rapid decrease in the effects of successive stimulations.

In no case, provided escape of current from the sympathetic electrodes to the adjoining *plexus ilio-lumbalis* was carefully avoided, was there any effect of the sympathetic stimulation (long or short) upon the muscle contractions whether they were a series of single contractions or of short tetani or whether the muscle was in good condition, fatigued or exhausted (more than two dozen experiments were made).

But if the strength of the current applied to the sympathetic was slowly increased, a point could be found in a few cases when, probably in consequence of spread of current, the muscle *suddenly* began to produce higher contractions in the rhythm of the root-stimulation. At this point apparently an escaped current was added to the rhythmic stimulations of the roots and strengthened their effects, though it was still too weak to excite the roots of itself.

Beyond that point the escaped current produced a simple tetanus of the muscle with its rhythmic contractions superposed on the tetanus. That these stronger contractions could not have been due to a sympathetic effect was proved by the fact that pinching the nerve below the electrodes did not alter the phenomenon and that the change came suddenly and not gradually.

*Action of adrenaline in the excised frog's sartorius muscle.*

The absence of effect of the sympathetic on the gastrocnemius described above, led me to make some observations to determine whether adrenaline has any obvious effect on survival frog's muscle—a point on which different observers have arrived at contradictory results. For this purpose, I took the sartorius muscle of the frog (*R. temp.*) immersed in Ringer's fluid in a Lucas chamber. The muscle was stimulated by means of electrodes placed at the opposite ends of the muscle. Induction currents were thus sent through the length of the muscle 15–30 times a minute. Whilst a series of contractions (fatigue curve) were being obtained, adrenaline was added to the Ringer's fluid to bring the adrenaline content in it to a degree varying from 1:500,000 to

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The roots were stimulated in the various experiments either each alone (in some cases also the 7th anterior root was found to be effective), or together. The sympathetic was stimulated with a suitable strength at almost every possible point of the fatigue curve of



stimulated during the fatigue curve of the anterior tibial muscle. The result, however, was a negative one.

Similar experiments were made on the frog's gastrocnemius muscle, when the circulation had ceased or nearly ceased. Stimulation of the sympathetic in this case was not found to have any effect. Finally, the fatigue contractions of the excised sartorius muscle of the frog (direct stimulation) was not found to be modified by adrenaline.

Thus, by the method employed, no evidence was found that the sympathetic affects striated muscle except by modifying the circulation.

I should like to thank the Council of Girton College, Cambridge, for the opportunity of studying in Cambridge, offered to me by their kind invitation.

My thanks are further due to Prof. Langley for all his kind advice and interest.

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# THE EFFECT OF CHANGES IN VENOUS BLOOD-PRESSURE ON THE RATE OF FLOW THROUGH, AND THE GASEOUS METABOLISM OF THE KIDNEY.

By E. W. H. CRUICKSHANK AND K. TAKEUCHI.

*(From the Physiological Laboratory, Cambridge.)*

THE present work was undertaken to determine the relation between the blood-pressure in the renal veins, and (1) the rate of flow of blood through the kidneys, and (2) the gaseous metabolism of the kidneys.

## *Methods.*

These experiments were carried out on cats under urethane anaesthesia (4 c.c. 25 p.c. urethane per kilo). Tracheotomy was performed and cannulae placed in the left carotid artery and right jugular vein.

*Dissection.* In order to have a clear field for the necessary manipulations in the kidney area, the cats were eviscerated, the methods described by Barcroft and Brodie<sup>(1)</sup> being followed, except that the evisceration was begun at the descending colon; the inferior and superior mesenteric arteries were cut between ligatures, the cardiac end of the oesophagus was cut between a double ligature and, after pressure upon the stomach and intestines to return to the circulation as much blood as possible, the coeliac axis and portal structures were tied and cut. This procedure, which entails no loss of blood, usually leaves the arterial blood-pressure higher than normal. Cats stand this manipulation well, the blood-pressure not varying more than 10 mm. Hg within half to one hour, provided the animal be kept warm.

The aorta was then tied just above the iliac bifurcation; the inferior vena cava dissected out well above the entrance of the renal veins; the left suprarenal vein, the lumbar veins and the ovarian vein tied. A loose ligature was put round the right renal veins and when the rate of blood flow from the left kidney only was required, the ligature was gently pulled upon, thus occluding the blood flow from the right kidney during the determination. After raising and massaging the hind legs of the animal the inferior vena cava was tied, as low down as possible, and a wide-mouthed cannula inserted. An arterial cannula was placed in the abdominal aorta and connected with a mercury manometer.

*Measurement of venous blood-pressure.* To the cannula inserted into the inferior vena cava, an S-shaped glass tube of 3 mm. internal diameter was attached; the tube was previously partly filled with 4 p.c. citrate solution. The height to which the solution rose in this tube could easily be measured, and after a reading, the blood was blown back into the venous cannula, a clip put on the vein and the cannula washed out.

The venous pressure was raised by placing a very small bull-dog clip on the inferior vena cava above the entrance of the left suprarenal vein. To measure large venous pressures the S-shaped tube was filled with citrate solution before being connected to the venous cannula.

*Measurement of the rate of flow of the blood.* A long 2 c.c. pipette, having an internal diameter of not less than 3 mm., the point of which had been cut off to eliminate obstruction to the flow of blood, was attached to the S-shaped tube. The pipette was held horizontally in a clamp, at a height which balanced the venous pressure and allowed the blood to oscillate in the upper horizontal part of the S-shaped tube. By means of a signal marker and a Jacquet chronographic clock marking fifths of a second on a smoked drum, the rate of flow was determined for 2 c.c. of blood, when the inferior vena cava was occluded above the clip. If the blood were not required for analysis it was blown back into the vein, the tube removed and the cannula immediately washed out with citrate solution. In several of our preliminary experiments we were troubled with clotting in the venous cannula. As defibrinated blood could not be used, we resorted to hirudin, but as the hirudin was made up in solution of .1 gm. in 25 c.c. it was manifestly introducing an abnormal factor to inject 150 to 200 mgms. of hirudin. In the experiments we give, no hirudin was used and with the immediate washing out of the cannulae with citrate solution upon withdrawal of blood samples there never was trouble with clotting. This is a matter of importance, for the accurate determination of the rate of blood flow is a *sine qua non* when the extent of the utilisation or production of a gas by an organ is under investigation.

*Collecting the blood samples.* Arterial blood was collected from the left carotid, the venous blood from the kidney was obtained by way of the inferior vena cava. All pipettes were drained with a saturated solution of neutral potassium oxalate. The estimation of oxygen was made by means of the Barcroft differential manometer, into the bottles of which were placed four drops of hirudin to prevent coagulation.

*The effect of increase in venous blood-pressure on urine flow.*

The early work concerning the effects of alterations in venous pressure upon the kidney and the blood flow through it has been summarised by Paneth(2). Early investigators were content with noting the speedy death of the animal and the presence of albumin casts and blood in the urine. That a diminution in the urinary flow was produced was demonstrated by Ludwig(3), in 1863, upon the isolated kidney by use of saline solutions. Paneth produced a diminution of flow with a venous pressure amounting to 2.8 cm. Aq. the normal pressure being 4.5 cm. Aq. Goll(4) in 1854, Munk and Senator(5) in 1888, and others, have shown that obstruction of the renal vein results in a diminution or cessation of urinary secretion. The only writer whose results are in opposition to this general result is Schwarz(6), who stated that the gradual compression of the renal vein of a kidney perfused with defibrinated blood resulted in an increase in urine flow. That a temporary increase in urine flow will follow very slight constriction of the vein was shown by Rowntree, Fitz and Geraghty(7), but they also showed that continued pressure ultimately resulted in a decrease of flow, the urine containing albumin casts and blood. Sollmann(8), using excised ox kidneys perfused both with saline and mixtures of saline and blood, has demonstrated that an increase of venous pressure some seventy-fold (*i.e.* from 0 to 70 cm. Aq.) decreased the urine flow almost 80 p.c. In our experiments we have invariably found that an increase in venous pressure causes a marked diminution in urine flow: an increase in pressure in the region of 100 p.c. causing a decrease in urine flow from one kidney, on an average of 75 p.c.

*The effect of increase in venous pressure on the rate of blood flow through the kidney.*

Sollmann, in his experiments upon excised kidneys prepared with saline, showed that increasing the vein pressure to 70 cm. Aq. produced a decrease in vein flow of 84 p.c. These experiments on the excised kidney perfused with saline or blood at pressures up to 140 cm. Aq. only indicate the mechanical adaptations which may obtain in a dead or dying organ. In order to arrive as accurately as possible at the mechanism in a living organ the organ must be *in situ* with its nerves intact and perfused with its own blood. During the altering conditions of the experiment there should be no addition of defibrinated blood or of gum saline, and further, the arterial blood-pressure should be main-

tained, and the kidney allowed to function in an environment approximating as closely as possible to the normal.

*The use of defibrinated blood.* Sollmann<sup>(8)</sup>, perfusing kidneys of dogs with defibrinated blood eight hours after excision, found that the venous flow increased and was often double that obtained with saline. He regarded two factors to be at work, viz. the factor of viscosity and an accelerating factor furnished by a dilatation of the renal vessels. He regarded this dilatation as active, stating that it exceeded the passive post-mortem dilatation of these vessels, that it probably involved the efferent arterioles, that is, a small constricted portion of the vascular bed being affected since the volume of the kidney was diminished, and that it was caused by a protein constituent of the serum. This increase commenced within five minutes of perfusion and the flow returned to about the normal level within 15 or 20 minutes. The ureter flow fell sharply with increase in vein flow and showed little tendency to recover. That urine flow is diminished and that the urine invariably contains albumin in artificially perfused kidneys has also been shown by Pfaff and Tyrode<sup>(9)</sup> and by Brodie<sup>(10)</sup>. This diminution Eichholtz and Verney<sup>(11)</sup> regarded as being due to a vaso-constriction caused by toxic substances in the defibrinated blood, which substances they state are removed by the passage of the blood through the heart and lungs. Several methods have been devised to obviate this vaso-constriction and secure an actively functioning organ. Bainbridge and Evans<sup>(12)</sup> used the isolated heart-lung preparation of Starling; Richards and Drinker<sup>(13)</sup> and Richards and Plant<sup>(14)</sup> secured actively secreting kidneys by means of a pump and hirudinised blood passed through the heart of the animal. Starling and Verney<sup>(15)</sup> devised the isolated heart-lung kidney preparation to secure this same end. By these means then the vaso-constriction or toxic effects were got rid of.

*Defibrinated blood with the kidney in situ.* In the results recorded in Tables I and II and shown graphically in Fig. 1, the addition of defibrinated blood is seen to produce a large increase in the rate of flow. From Table I it will be seen that the average normal rate of flow was 43.5 c.c. per min.; that 50 minutes after defibrinated blood had been added in quantities just sufficient to maintain the arterial blood-pressure as nearly constant as possible, the rate of flow was 60 c.c. per min. and that 30 minutes later it had increased to 75 c.c. per min. That is, in 1 hour 20 minutes the rate of blood flow through the kidney had increased 70 p.c. of the normal, and 1 hour 45 minutes from the commencement of adding defibrinated blood the rate of flow was 56 c.c. per

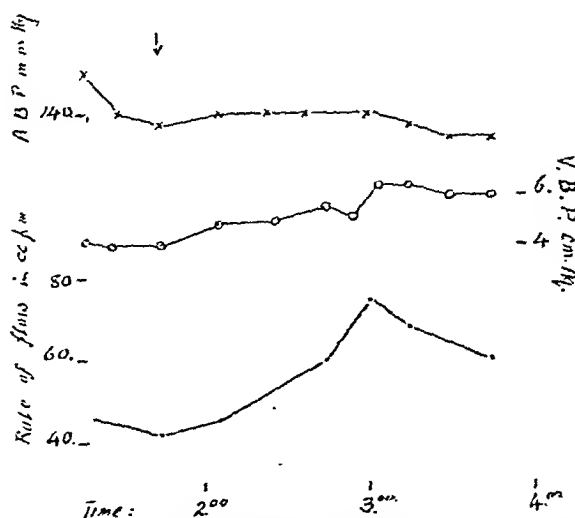
TABLE I. Effect of adding defibrinated blood: one kidney in circulation: wt. of kidney = 16.0 grms.

Time	A.B.P. mm. Hg.	V.B.P. cm. Aq.	Rate of flow c.c. per min.	Blood taken
1.30	160	4.2	46.2	—
1.35	140	4.2	42.8	5 c.c.
1.45	134	4.2	41.6	—
1.50	Commenced to add defibrinated blood			
2.10	140	4.6	46.2	—
2.25	140	4.8	—	—
2.45	140	5.6	60.0	5 c.c.
2.48	140	5.0	60.0	—
3.10	140	6.3	75.0	—
3.15	136	6.3	66.6	5 c.c.
3.40	130	6.0	60.0	5 c.c.
3.45	130	6.0	58.0	—

Three similar experiments were performed: one with both kidneys; two with increased venous pressure. Defibrinated blood was added in small quantities by way of a cannula in the external jugular vein. Total amount of blood added from 1.50 p.m. to 3.40 p.m. was 100 c.c.

TABLE II. Effect of adding defibrinated blood with increase in venous blood-pressure, both kidneys: wt. R. = 17.5 grms.; L. = 17.0 grms.

1.30	166	3.3	30.0	5 c.c.
2.00	162	3.3	39.8	—
2.30	154	3.3	42.8	5 c.c.
2.40	140	6.0	(Clip on I.V.C.)	
3.00	Commenced adding defibrinated blood			
3.15	148	4.5	62.8	5 c.c.
3.40	140	5.2	30.0	(R. kidney out)
4.00	148	4.4	60.0	5 c.c.
4.02	150	5.0	59.6	—
4.45	142	8.5	Venous pressure increased	
5.00	144	9.0	66.6	5 c.c.
5.15	146	7.0	66.6	—

Fig. 1. Effect of addition of defibrinated blood upon the blood flow through the kidney *in situ*.

min., approximately a 30 p.c. increase. It must be remembered that this result is obtained in the eviscerated animal; the splanchnic mechanism has been removed and it therefore indicates that there is an active dilatation. Similar results have been obtained with both kidneys in circulation (Table II). From a normal flow of 30 c.c. per min. the rate had increased at the end of 1 hour to 42.8 c.c. per min., an increase of 42 p.c. One hour later the rate had increased by approximately 100 p.c., and an increased rate of flow varying from 40 to 100 p.c. was maintained, until the end of the experiment which lasted  $4\frac{3}{4}$  hours.

It seems then highly probable that in the kidney *in situ* the effect of the addition of defibrinated blood which passes through the heart and tissues of the animal, is to cause a definite vaso-dilatation and not a vaso-constriction, as has been shown to obtain in the isolated kidney perfused with defibrinated blood by means of a pump. That this increase in flow is not due to a decrease in the viscosity of the blood is shown by the fact that the increase occurs during the first half-hour of the injection of defibrinated blood, during a period in which the amount of blood added never exceeded 30 c.c.

The fall of blood-pressure which results upon first taking about 5 c.c. of blood for estimations is restored usually within 2 or 3 minutes. As the experiment proceeds, however, the restoration is not complete, largely because of the loss of the adaptation mechanism of the splanchnic area. After 30 to 45 minutes from the taking of the first samples there is a progressive fall in blood-pressure; there is no hæmorrhage but there must be a loss of fluid to dependent parts, a loss of fluid which during the first hour of the experiment, that is, from the completion of the operative work, is very slight and with good experimental conditions causes little alteration in the blood-pressure: the alteration amounts to not more than 2 to 3 p.c. change, a change which has no effect on the venous pressure and none on the rate of blood flow.

In view of the facts that defibrinated blood in comparatively small quantities definitely alters the rate of flow, that the alterations in blood-pressure during the first 30 or 40 minutes are very small, and that it is essential to have a circulating medium, the percentage oxygen capacity of which should be constant, we decided to run our experiments in periods of 30 minutes' duration, and to restore the blood-pressure at the end of the period after taking the blood samples for the necessary estimations. During the second period the blood-pressure usually fell slightly, about 5 to 10 p.c. of the original pressure. If the change approached the latter figure a little defibrinated blood was added, but usually this did not

amount to more than 15 c.c. throughout the 30 minute period. Two such periods were all that we required and we believe that with a maintained blood-pressure and the addition of such a small amount of defibrinated blood during the subsequent period, we have secured conditions sufficiently comparable to give us a close approximation to the mechanism involved.

Alterations in arterial blood-pressure occasion changes in blood flow and in venous pressure, therefore, in any determination of the blood flow through an organ and of its relation to venous pressure the arterial blood-pressure must be kept as nearly as possible at a definite level. The level which we have taken is that occurring after the withdrawal of the first samples of blood; this had no effect usually on the blood-pressure.

The changes in the rate of flow occasioned by alterations in venous pressure are shown in diagrammatic form in Figs. 2 and 3 and in Table III. There is invariably a decrease in the rate of flow through the kidney. The average of seven experiments shows that for an increase of 65 p.c. in venous pressure the rate of flow through the organ is diminished by 35 p.c.

TABLE III. Effect of increased venous pressure on blood flow through the kidney and on its gaseous metabolism. Weight of left kidney 10.5 grms.

Time	A.B.P. in mm. Hg.	V.B.P. in cm. Aq.	Rate of flow c.c. per min.	O <sub>2</sub> used per c.c.	O <sub>2</sub> used per min.
12.50	150	4.5	30.0	.0228	.647
1.00	150	3.5	27.2	—	—
1.15	120	8.8	(Clip on i.v.c.)	—	—
1.30	120	—	—	—	—
2.00	122	8.5	20.4	.0281	.594
2.30	114	9.3	11.30	.0551	.629*
3.30	110	11.7	15.20	.0374	.569

\* 15 c.c. defibrinated blood added slowly, from 2.40.

Eight such experiments were performed with similar results. Experiments with both kidneys in function gave results strictly comparable to the above.

*The effect of changes in venous pressure upon the  
gaseous metabolism of the kidney.*

From the tables and diagrams already referred to it will be seen that the oxygen utilised per cubic centimetre of blood flowing through the kidney varies inversely with the rate of blood flow through the organ. This relation is maintained only so long as no defibrinated blood is added and the venous pressure causes no occlusion of the renal vein. With the addition of blood the flow increases, returning and going beyond the original normal rate, the utilisation of oxygen per c.c. of blood falls to the normal and in some cases, below it. The utilisation of oxygen



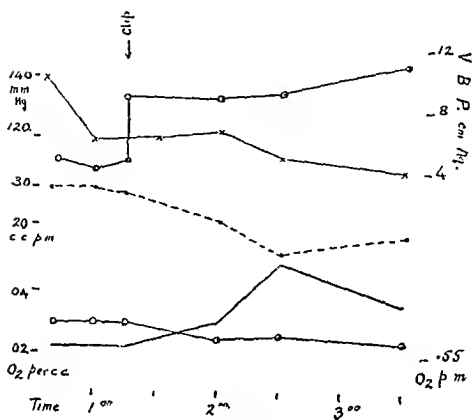


Fig. 2 Absolute changes.

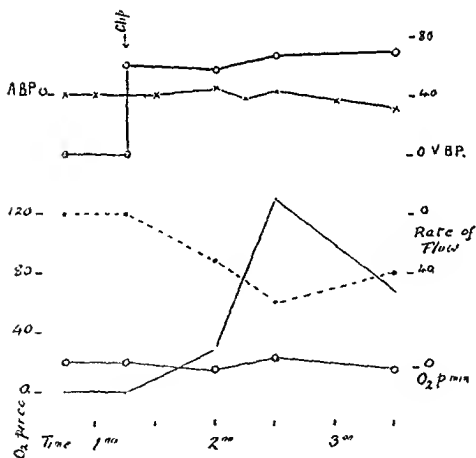


Fig. 3. Percentage changes.

Figs. 2 and 3. Effect of increased venous pressure on blood flow and oxygen utilised.

per min. is almost constant; it is only after the first 2 hours of the experiment that any abnormal figures for oxygen utilisation are obtained, by which time the condition of the animal has become definitely impaired. The curve of oxygen utilisation per min. invariably approximates to a straight line. As far as we have gone this would indicate that the kidney is mechanically adapting itself to the altered conditions of blood flow and therefore of oxygen supply. What the ultimate result would be of increasing the venous pressure beyond the limits already shown, is of no practical importance, since a maximal pressure would mean a zero blood flow and a rapidly dying organ. That an increase of pressures amounting to from 80 to 100 p.c. for 1 to 2 hours does not materially affect the organ is shown by the return to normal blood flow and oxygen utilisation in several experiments. It is quite possible that the kidneys could maintain a normal function against an increase of venous pressure of some 30 p.c. provided that the conditions producing the increase did not in any way alter the oxygen percentage saturation of the arterial blood.

These experiments would indicate that during the period of the experiment, 2-3 hours, the kidneys are not rendered anoxæmic by anything up to a 60 p.c. decrease in the rate of flow of blood through them.

In Table IV, which gives a comparison of oxygen used per c.c. of blood with the changes in the rate of flow, it will be seen that with a

TABLE IV. Comparison of the percentage changes in the rate of blood flow, with those of oxygen utilisation per c.c. of blood flowing through the kidney.

With increase in flow due to the addition of defibrinated blood		With decrease in flow due to increase in venous pressure without adding defibrinated blood	
Percentage increase in rate of flow	Percentage decrease in O <sub>2</sub> used per c.c.	Percentage decrease in rate of flow	Percentage increase in O <sub>2</sub> used per c.c.
55	30	40	36
12	44	34	52
39	57	60	140
74	55	28	65
30	19	17	15
—	—	8	11
42.0	41.0	31.2	53.1

normal kidney the increased rate of flow due to the addition of defibrinated blood results in a decrease in the amount of oxygen taken from each c.c. of blood. The average of five experiments would show that the adaptation is purely mechanical, whereas in a condition which be-

comes progressively abnormal there is some indication that the organ adapts itself to the abnormal conditions by an utilisation of oxygen greater than that which a merely mechanical alteration in the rate of blood flow would demand. Where the utilisation per min. falls, then, one may correctly assume that the kidney was failing to function normally. In most of these experiments there is a slight fall in the oxygen percentage saturation of the blood; this fall does not amount to more than a drop from 96 to 80 p.c. for arterial blood and from 82 to 61 p.c. for venous blood. Such a fall still leaves the organ a considerable margin of safety.

#### CONCLUSIONS.

1. Defibrinated blood circulating through the heart and kidneys *in situ* produces vaso-dilatation; this vaso-dilatation is progressive and depends upon the amount of defibrinated blood used; it usually attains its maximum within 1 and 1½ hours.

2. An increase in the blood flow produces a proportionately inverse utilisation of oxygen per c.c. of blood flowing through the organ.

3. An increase in the venous pressure in the renal veins produces a proportionate decrease in the blood flow through the kidneys, with an increase in oxygen used per c.c. of blood.

4. An increase in venous blood-pressure up to 100 p.c. does not necessarily render the kidney anoxæmic; this is shown by the average figures for oxygen percentage saturation at the end of the experiments, viz. arterial blood 88.8 p.c., venous blood from the kidney 69.9 p.c.

5. For a certain time, varying from 1 to 2 hours, the kidneys under conditions of increased venous pressure amounting to from 50 to 100 p.c., utilise an amount of oxygen per c.c. of blood, which, in relation to the rate of blood flow, gives a constant utilisation of oxygen per min.

6. An increase of pressure in the renal vein varying from 50 to 100 p.c. reduces the flow of urine from approximately 12 c.c. to one of 4 or 3 c.c. per hour for one kidney, i.e. a 66.6 to 75 p.c. decrease.

7. The average oxygen consumption of the cat's kidney per gram of kidney per min. is .06 c.c.

We wish to express our thanks to Mr Barcroft for suggesting the problem and for his continued interest in a piece of work which was beset with certain technical difficulties.

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# THE RELATION OF THE THYROID GLAND TO THE ACTION OF INSULIN.

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AN experimental investigation of the connection between the thyroid gland and carbohydrate metabolism was first attempted by Eppinger, Falta and Rudinger(1) in 1905, who claimed that in the absence of the gland, glycosuria was not produced by the injection of adrenaline into dogs. Controversy on the truth of this, and of the allied claim that adrenaline hyperglycæmia is less in the thyroidectomised than in the normal animal, still continues, the latest paper being that of Geiger(2) who denies that there is evidence for either. An important observation was made by Cramer and Krause(3), who found that the addition of thyroid gland to the diet of cats and rats resulted in the total disappearance of glycogen from the liver, their work has since been confirmed by Kuriyama(4). Recently, while the investigation described in this paper was in progress, Bodansky(5) has recorded that the hypoglycæmic reaction to insulin is greater in sheep after thyroidectomy, and Ducheneau(6) has found that the lethal dose of insulin is smaller for thyroidectomised than for normal rabbits. Macleod(7) originally put forward the hypothesis that during the hypoglycæmia produced by insulin the liver discharges glycogen in an effort to raise the blood sugar to the normal level. Lately this has been disputed by Cramer(8), who interprets the diminution of liver glycogen observed by Dudley and Marrian(9) as due to diminished glycogen formation. Macleod's conception is supported, on the other hand, by Burn's observation(10) that ergotamine, which paralyses the mobilisation of sugar from the liver by sympathetic impulses and adrenaline, also intensifies the hypoglycæmia produced by insulin. In the following paper we present the results of experiments made to test this hypothesis further, by studying (1) the effect of cutting the splanchnic nerves on the effect of insulin, (2) the relation between the hyperglycæmia produced by adrenaline and the hypoglycæmia produced by insulin in different rabbits, and (3) the effects on both these reactions of thyroidectomy and thyroid feeding.

*Section of both splanchnic nerves.* The operation of dividing both splanchnic nerves below the diaphragm is one which is better tolerated by the cat than by the rabbit. The observations were therefore made on cats, and owing to the difficulty of making repeated blood sugar determinations, the degree of hypoglycæmia produced by an injection of insulin was judged by the symptoms, which appear with greater regularity, and less abruptly, than in the rabbit. The stages observed were (1) drowsiness and disinclination to walk; (2) increasing ataxia; (3) inability to walk, the cat lying on its side apparently unconscious; (4) convulsions of a violent character.

Each animal was kept on an ample diet of meat with bread and milk. Before an observation it received no food for 16 hours. It was then weighed, and a known amount of insulin was injected under the skin. Starting from doses which produced no symptoms, the effect of increasing amounts was observed. From three to ten observations were made on each animal, both before and after the section of the splanchnic nerves. These were divided in two operations, at an interval of 8 to 10 days, the operations being performed under deep ether anæsthesia, with full aseptic precautions, by the lumbar route. A period of 10-14 days was allowed to elapse after the second operation before the second series of observations with insulin was begun, the immediate effects of operation having disappeared completely by this time. The results obtained are shown in Table I.

TABLE I.

	Weight kgm.	No. of observations	Severe symptoms produced by insulin dosage of mgms. per kgm.	No symptoms produced by dose of mgms. per kgm.
Cat A when normal	2.75	10	.2	.14
after operation	2.80	6	.13	.10
Cat B when normal	2.2	5	.30	.20
after operation	2.45	3	.10	.05
Cat C when normal	2.1	3	.20	.15
after operation	2.8	3	.10	.05

The table gives the weights of each cat before and after the operations, and the number of observations made in order to arrive at the dosage necessary to produce on the one hand, severe symptoms of hypoglycæmia and, on the other, no definite effect. "Severe symptoms" indicates the condition in which the cat is apparently unconscious and lies on its side. In the case of each animal, a dose less than that which was without effect before, produced severe symptoms after the operations. It is evident that the removal of the central control of the liver and

suprarenals increased the hypoglycæmia produced by a given amount of insulin, a result consistent with the view that the compensatory discharge of glycogen from the liver normally attending the insulin effect is in part produced by impulses passing down the splanchnic nerves.

*The relation of adrenaline hyperglycæmia to insulin hypoglycæmia.* The extent of hyperglycæmia produced by a given amount of adrenaline varies widely in different rabbits, although these receive the same diet for many weeks before the tests are made. Similarly, the hypoglycæmia produced by a given amount of insulin varies in different rabbits. The variation in these two reactions may be connected, for if the second be limited by glycogenolysis prompted by stimulation of sympathetic nerve endings, then it should be smaller in those animals in which the hyperglycæmia produced by adrenaline is relatively large. The following examples illustrate that this is the case.

*Exp. 1.* Two rabbits, each weighing 3 kgm. received injections (s.c.) of .5 mgm. adrenaline. Blood sugar determinations were made by the Shafer-Hartmann method at hourly intervals after injection.

	Initial B.S.	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	Average
Rabbit 1	.12 p.c.	.33	.33	.35	.25	.16	.29
Rabbit 2	.10 „	.20	.22	.24	.18	.13	.19

The hyperglycæmic reaction was much greater in Rabbit 1.

The injection of 1.6 iugm. per kgm. of a sample of insulin hydrochloride into Rabbit 1 produced no symptoms, and the lowest blood sugar observed was .06 p.c. The injection of less than one-tenth this amount, namely .15 mgm. per kgm. into Rabbit 2, produced convulsions, and the injection of .1 mgm. per kgm. reduced the blood sugar to .04 p.c. The hypoglycæmic reaction was much smaller in Rabbit 1.

*Exp. 2.* Five rabbits were taken, all of about 2 kgm. weight. The hyperglycæmic reaction to an injection of .4 mgm. adrenaline was determined for each by taking five samples of blood at hourly intervals after the injection. From the average of these the initial blood sugar was deducted, and the difference used as a measure of the reaction. The hypoglycæmic reaction to the injection of .2 mgm. insulin hydrochloride was determined by a similar procedure. The results were as follows:

	Hyperglycæmic reaction	Hypoglycæmic reaction
Rabbit 3	.073 p.c.	.063 p.c.
„ 4	.135 „	.023 „
„ 5	.126 „	.022 „
„ 6	.149 „	.036 „
„ 7	.113 „	.018 „

The hyperglycæmic and the hypoglycæmic reactions of Rabbits 4, 5, 6 and 7 were respectively alike. The hyperglycæmic reaction of Rabbit 3

was much less than that of the others, while the hypoglycæmic reaction was greater; at the second hour the blood sugar was  $\cdot 026$  p.c., whereas, in the other four animals it was  $\cdot 06$  p.c. or more; at the third hour Rabbit 3 had convulsions when the blood sugar of the others was  $\cdot 072$  p.c. or more.

*The relation of the thyroid hormone to the reactions.*

(a) *The effect of thyroidectomy.* Several observers have recorded (see Geiger(3)) that after thyroidectomy the hyperglycæmia produced by a given amount of adrenaline is less. We have published(11) in a preliminary note figures showing that the hypoglycæmia due to a given amount of insulin is greater, confirming the records of Bodansky(5) and Ducheneau(6). We have now carried out in three rabbits observations both on the insulin hypoglycæmia and the adrenaline hyperglycæmia, before and after thyroidectomy.

*Exp. 3.* The rabbits were maintained under constant conditions for several weeks before and during the experiments.

A. *Insulin hypoglycæmia.* In each case an attempt was made to determine the minimal convulsive dose of a given preparation of insulin for each rabbit. The level of the blood sugar was also followed at hourly intervals during the experiments. Each figure in the following table represents the results of from three to six separate tests.

	Minimal convulsive dose before thyroidectomy mgm. per kgm.	Minimal convulsive dose after thyroidectomy mgm. per kgm.	Increase in sensitiveness
Rabbit 1	Greater than 1.6	$\cdot 25$	More than 6 times (Probably 9 times)
" 8	1.2	$\cdot 25$	About 5 times
" 2	0.15	$\cdot 05$	3 times

The lowest blood sugar recorded following the injection of 1.6 mgm. per kgm. of insulin in Rabbit 1 before thyroidectomy was  $\cdot 061$  p.c., a figure considerably above the convulsive level; probably a dose of 2.4 mgm. would have produced convulsions. Had this been the case the increase in sensitiveness following thyroidectomy would have been nine to ten times. The injection of  $\cdot 25$  mgm. after the thyroidectomy reduced the blood sugar to  $\cdot 029$  p.c. in this rabbit.

The lowest blood sugar observed after the injection of 1.2 mgm. per kgm. insulin in Rabbit 8 before thyroidectomy, was  $\cdot 042$  p.c.; consequently, although this dose did not produce convulsions it was near the convulsive level.

B. *Adrenaline hyperglycæmia.* In each case  $\cdot 5$  mgm. adrenaline was



injected. In the following table the *average* blood sugar during the five hours following the injection is recorded.

	Average hyperglycaemia		Difference
	Before thyroidectomy	After thyroidectomy	
Rabbit 1	295 p.c.	235 p.c.	060
" 8	232 "	207 "	025
" 2	192 "	183 "	009

The difference in the adrenaeline hyperglycaemia before and after thyroidectomy is small, the figures represent the average of five determinations, however, and therefore the difference, even in the case of Rabbit 2, is probably real. Comparing the two tables, the greatest increase in sensitiveness to insulin occurred in Rabbit 1, in which there is the greatest reduction in the hyperglycaemic reaction, similarly, the least increase in the hypoglycaemic reaction was in Rabbit 2, which showed the least reduction of the hyperglycaemic reaction.

(b) *The effect of thyroid feeding* Experiments have been carried out in which a dried preparation of thyroid gland (Burroughs, Wellcome and Co.) was added to a diet consisting of a mixture of boiled potatoes and crushed oats. Of this a rabbit, of weight 2 kgm. received 100 gm. a day, containing the powdered substance of four tablets, representing 1.2 gm. of fresh gland, according to the manufacturer's indication, occasionally this amount was increased to 1.8 gm. of fresh gland. The effect of this feeding was to cause the animals to lose weight. Thus, in 20 days, a rabbit starting at 2.2 kgm. fell to 1.35 kgm.

The extent of the hypoglycaemic reaction to insulin rapidly diminishes after a few days' thyroid feeding. Fig. 1 shows the initial reaction of a thyroidectomised rabbit, to an injection of insulin which produced convulsions, and also the much smaller reaction after 8 days' thyroid feeding to five times this dose of insulin. The injection of ten times this dose did not produce any symptoms. Other examples of similar effects are shown in the following records.

Rabbit 9 Initially 25 mgm. per kgm. insulin produced convulsions. After 8 days' thyroid feeding, the injection of 75 mgm. per kgm. produced a hypoglycaemia in which the blood sugar did not fall below 0.6 p.c. After 12 days the injection of 1 mgm. per kgm. did not produce convulsions.

Rabbit 10 Initial injection of 25 mgm. per kgm. produced convulsions. After 8 days' thyroid feeding 1 mgm. per kgm. insulin produced a fall of blood sugar in which the lowest value was 0.3 p.c.

These results indicate that the presence of large amounts of the thyroid hormone in the circulation enables the organism to prevent the

occurrence of severe hypoglycæmia, in spite of the injection of relatively large doses of insulin.

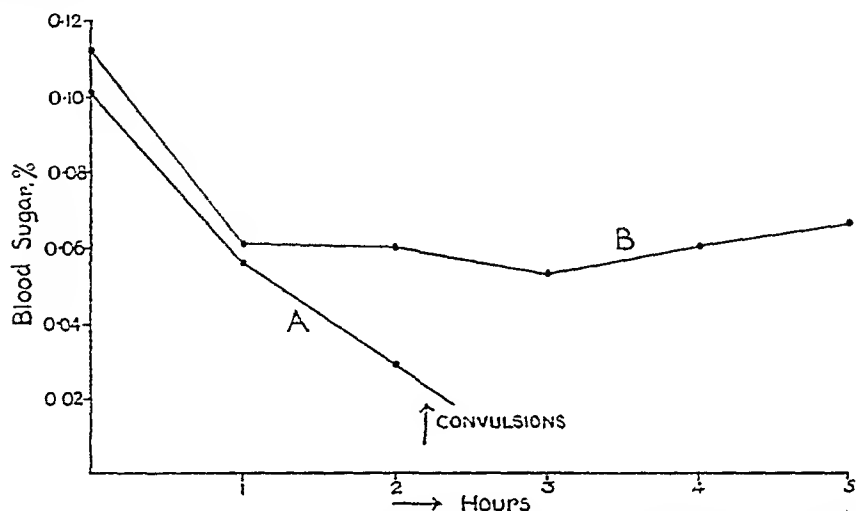


Fig. 1. Curve A shows the hypoglycæmic reaction of a thyroidectomised rabbit to .05 mgm. per kgm. of insulin. Curve B shows the reaction of this rabbit to five times the dose after 8 days' thyroid feeding.

After thyroid feeding there is an increase in adrenaline hyperglycæmia, which is more uniform than the decrease produced by thyroidectomy. We have observed this increase with intravenous as well as with subcutaneous administration of adrenaline. The following are examples:

		Reaction when normal	Reaction after thyroid feeding
Rabbit 11	.05 mgm. adrenaline i.v.	.02 p.c.	.04 p.c.
" 12	.2 " s.c.	.03 "	.09 "

The reaction in Rabbit 11 is the difference between the average blood sugar during 1 hour after the injection (three samples of blood) and the initial blood sugar; the change observed occurred after 7 days' thyroid feeding. The reaction in Rabbit 12 is the difference between the average blood sugar during 5 hours after the injection (five samples of blood) and the initial blood sugar; the change occurred after 14 days' thyroid feeding.

By prolonging thyroid feeding beyond 10-14 days we have found that the hypoglycæmic reaction to insulin after the initial great reduction, once more increases. Fig. 2 a shows the changes in the hypoglycæmic reaction of a rabbit to a given amount of insulin during 18 days' thyroid feeding. The reaction was measured by taking the average figure of five blood sugar determinations at hourly intervals and sub-

tracting it from the initial blood sugar figure. After becoming progressively less in intensity and duration, the hypoglycæmia afterwards

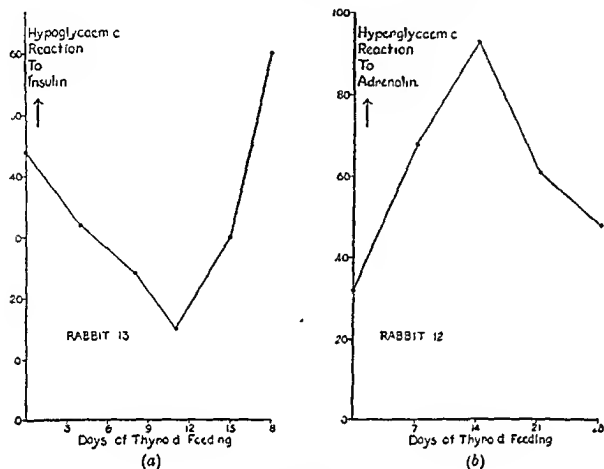


Fig 2 See text

increased so that on the eighteenth day the rabbit had severe convulsions following the injection, and died, in spite of subcutaneous injection of sugar. Fig. 2 *b* shows, again, that the adrenalin hyperglycæmia undergoes changes inversely corresponding to the changes in the insulin hypoglycæmia, as thyroid feeding progresses, after having increased during the earlier period it decreases during the later period of thyroid feeding. The hyperglycæmic reaction in the figure is expressed as the difference between the average of the five determinations of the blood sugar percentage, determined at hourly intervals after the injection, and the initial blood sugar.

#### *Changes in liver glycogen during thyroid feeding.*

Since Cramer and Krause (3) have shown that thyroid feeding leads to the disappearance of liver glycogen, and since we suppose that the output of sugar from the liver is the most important factor limiting the hypoglycæmia produced by insulin, it was important to determine the course of the disappearance of the glycogen store during the changes in the hypoglycæmic reaction.

that every tested condition, natural or artificial, which has been shown to enhance the hyperglycæmic response to adrenaline has been observed to depress, in a more striking manner, the hypoglycæmic response to insulin, while conditions which impair the response to adrenaline, accentuate that to insulin.

It seems reasonable to suppose that the prolonged stimulation of the liver cells by sympathetic impulses or adrenaline when evoked as a reaction to the effect of insulin, is a more truly physiological process than the more evanescent and intense effect produced by sudden injection of adrenaline itself, and consequently more easily modified by changing the amount of thyroid hormone in circulation. As to the precise mechanism of this compensatory action of the liver, we have little evidence to offer. That it is in part produced through the central nervous system appears to be demonstrated by the increased effect of insulin when the splanchnic nerves are divided. It cannot be supposed, however, that this operation annuls the reaction completely. More peripheral sites of stimulation must exist. But our results afford no evidence as to the nature of the stimulus. Whether on nerve centres, suprarenal glands, or the liver cells themselves, the stimulus, so far as our evidence goes, may be due either to lack of dextrose or to excess of insulin itself in the blood.

The view that the liver thus modifies the insulin hypoglycæmia, by accelerated output of dextrose, has been opposed by Cramer (8), who advances two principal arguments against it. He states, in the first place, that hyperthyroidism empties the liver of glycogen, while diminishing sensitiveness to insulin. In the second place, he suggests that in hyperthyroidism, though the liver is depleted, the blood sugar is abnormally high. It appears to us that our experiments dispose of these difficulties. The effect of thyroid feeding on carbohydrate metabolism in the rabbit is relatively slow in development. It is easy to distinguish, in this species, the earlier stage, in which the accelerated glycogenolysis diminishes the response to insulin, as suggested by Cramer; but at this stage the liver has still an adequate glycogen store. Similarly, it is true that hyperthyroidism may be accompanied by hyperglycæmia; but our evidence does not support Cramer's suggestion that under these conditions the liver glycogen is already exhausted. On the contrary, when that stage of the effect has been reached, we find that the animal passes into a spontaneous, acute and fatal hypoglycæmia.

## SUMMARY

1 Section of both splanchnic nerves in the cat increases the hypoglycæmic reaction to insulin

2 The hyperglycæmic reaction to adrenaline varies in different rabbits as does the hypoglycæmic reaction to insulin, in those animals in which the former is large, the latter is small, and *vice versa*

3. Thyroidectomy diminishes the hyperglycæmic reaction to adrenaline and increases the hypoglycæmic reaction to insulin

4 Thyroid feeding, so long as the glycogen store in the liver is not diminished, increases the hyperglycæmic reaction to adrenaline and decreases the hypoglycæmic reaction to insulin

5 When, as a result of prolonged thyroid feeding, the liver glycogen disappears, the hyperglycæmic reaction to adrenaline diminishes and the hypoglycæmic reaction to insulin increases

We wish to thank Dr H H Dale for his help with the majority of the operations.

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## ON THE CONDITIONS OF RESPIRATION WITH THE CHEST OPEN. BY K. TAKEUCHI.

*(From the Physiological Laboratory, Cambridge.)*

THE following experiments were undertaken as a preliminary to some other work in order to ascertain whether the conditions of oxygen exchange could be kept at normal level with the chest open for considerable periods of time. The points aimed at were:

- (1) That the animal should live up to nine hours (under urethane).
- (2) That the arterial blood should be efficiently oxygenated.
- (3) That the total ventilation should be normal.

Firstly, some preliminary enquiries were made into the cause of failure of the usual forms of apparatus. Frequently one of two things happens when the chest is opened: (1) the arterial blood is insufficiently oxygenated, or (2) the total ventilation has to be increased much beyond what would be sufficient to oxygenate the blood when the chest is closed. In either case the conditions are abnormal. Even this unsatisfactory state is not maintained, and usually a condition supervenes in which, in spite of a greatly increased total ventilation, the arterial blood darkens, ultimately with fatal results. In the first forms of apparatus which we used death took place usually about three hours after the chest was opened. Our first object was to ascertain the cause. This was found by histological examination to be a high degree of emphysema. In order to inflate the whole of the lungs with the chest open, parts become grossly over-distended.

At this point it may be well to describe the form of artificial apparatus which we used. It was prompted chiefly by economy, and given a supply of compressed air and the necessary power the remaining expenses are very small; for the principle I do not claim originality, but the details, as will be seen, are all important.

The principal part of the apparatus is a glass three-way tap, as shown in the figure, which renders valves unnecessary. One way *B* is connected to the tracheal cannula *C*, another *D* to the compressed air, and the third is open to discharge the expired air. The stopper of this tap has two holes to connect the first way to the second or to the third, *E*. When *B* and *D* are connected, the lung is inflated and when *B* and *E* are connected, the expiration takes place. The stopper is turned by a motor and the inspiration and expiration take place alternatively.

A large empty bottle, *F*, of about 10 litres capacity, is fitted between the compressed air and the tap, to maintain a constant pressure of inspired air. To this bottle three tubes are fitted, one, *G*, from the tap, the second, *H*, from the stopper of compressed air supply and the third, *K*, leads to a tube which dips below the surface of water in a cylinder *L*, sufficient air being let in to maintain an air overflow through the tube and so keep a constant pressure in the bottle. The pressure of water in the bottle is equal to the height of water from its surface to the opening of the tubing. The pressure can be changed by moving the tubing up or down in the water. The diameter of the tubing *K* should be large, relatively to *H* and *G*, if not, the pressure in the bottle cannot be kept constant. I used a rubber tubing of 1.8 cm. diameter and the height of the water was between 8-12 cm.

A T-shaped glass tubing *M* is fitted on one part of the tubing between the stopper of compressed air and the bottle, in case one should wish to use gases other than air, for example oxygen or nitrogen for inspired air. And another T-shaped glass tubing *N* is fitted between the bottle and the valve; here samples of inspired air may be taken for analysis. The side limbs of *M* and *N* are ordinarily closed by screw-clips.

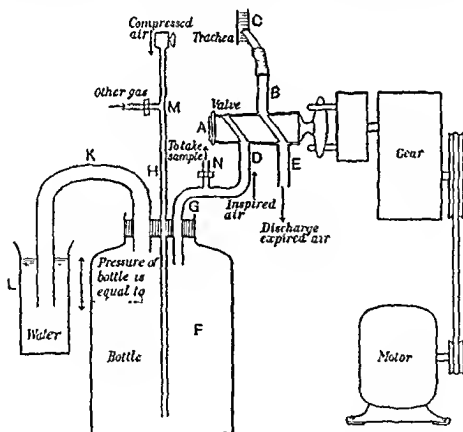


Fig. 1.

To revert to the cause of emphysema so long as an ordinary three-way tap is used, even if the bore be large, the period during which air is entering the lung will only be a small fraction of the whole time taken

for the tap to revolve. If the number of respirations per minute be normal, the actual time taken for inspiration and expiration respectively will be relatively small as compared with the time taken during natural respiration; for with such a tap during most of the cycle the tap is completely shut. In order, therefore, to inflate and deflate the lungs in so short a time there must be a much greater difference of pressure between the lung and the outside air than is normally the case. The air is blown in at a high pressure and must attain an abnormal pressure in the lung in order to escape quickly when the opportunity occurs. Hence the cause of the emphysema. A special tap was found to give satisfactory results, the best of several which I used had a stopper 5.5 cm. long and 2.2 cm. diameter at the thicker end. The holes were of 4 mm. diameter and at both ends the holes were enlarged so as to increase the times available for expiration and inspiration three- or four-fold. The stopper is shown in Fig. 2.

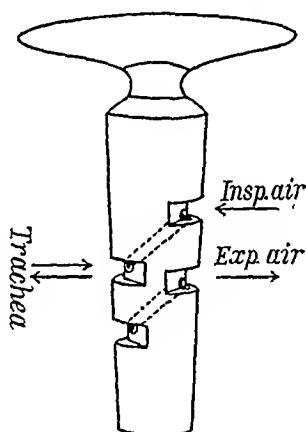


Fig. 2

When the stopper is kept revolving at a constant speed the slots insure that during almost half the time of revolution the inspired air supply has access to the trachea, whilst during almost half the revolution the trachea has access to the expiratory-tube. A comparison of Exps. 1 and 2 shows the difference between an ordinary three-way tap with a bore of 1.5 mm. and the tap described above. In the former case the pressure of the inspired air was 20 cm. of water, in the latter 8 cm. The former experiment ended fatally after four hours. In the latter the animal was in good condition after nine hours. The total ventilation was much the same in both cases.

*Exp. 1.* Cat, 2.5 kg. (urethane 1 grm. per kilo). Artificial respiration by my method but using a smaller tap with holes of 1.5 mm. diameter. Pressure of air in the bottle was 20 cm. water height. Frequency of rotation of valve was 52 per minute.

	After opening the chest	Vol. of expired air per min. in c.c.	O <sub>2</sub> consumed per min.	CO <sub>2</sub> given out per min.
1.	1 h. 10 m.	445	8.4	6.6
2.	2 „ 35 „	430	9.0	10.0
3.	3 „ 27 „	430	6.7	4.4
Death.	4 „ 10 „	—	—	—

At the end of the third hour the metabolism had fallen off considerably, whilst the cat only survived the opening of the chest by four hours and ten minutes.



*Exp 2* Cat, 1.5 kg (urethane 1 gm per kilo) Artificial respiration using a larger valve with holes of 4.1 mm diameter Pressure of air in the bottle was 8 cm water height Frequency of rotation of the valve was 0.2 per minute

	After opening the chest	O <sub>2</sub> p c of expired air	Vol of expired air in c c	O <sub>2</sub> taken in per min
1	1 h 0 m	19.18	560	10.0
2	2 „ 35 „	18.85	425	9.0
3	4 „ 0 „	19.32	590	9.7
4	5 „ 40 „	18.82	450	9.7
5	6 „ 33 „	18.82	535	11.4
6	7 „ 18 „	18.37	430	11.1
7	7 „ 55 „	18.86	435	9.2
8	8 „ 26 „	19.00	455	9.0
9	8 „ 55 „	18.80	510	10.9

Nine hours after opening the chest the animal was still alive and its metabolism was well maintained

In our endeavour to obtain constancy in the fundamental respiratory conditions, though with an open chest, we may commence with the consideration of the body temperature. If the body temperature is kept constant the quantity of oxygen used per minute should also be constant. In all experiments, therefore, the body temperature was kept constant within half a degree, and the oxygen consumption was only a little irregular and was well maintained, showing on the one hand that the animal remained in good condition and on the other that there was no struggling for want of oxygen.

*The total ventilation* With 5 cm water pressure and 33 to 35 artificial respirations per minute, the total ventilation is scarcely changed from the natural respiration, but with Muller's valves with only 3 cm pressure, the total ventilation is markedly cut down. A good pressure to use is 5 cm, this gives ventilation of 500-600 c c per minute, which is not less than a cat with valves frequently respire and may be considerably in excess of the ventilation of a cat which is breathing very quietly as was that of *Exp 2*. Three centimetres' pressure may cut down the total ventilation very much. These two points are illustrated by the following data.

		Rate of respiration	Total ventilation	Pressure of respired air
<i>Exp 3</i>	Natural with valves	25-26	585-625	—
	Artificial	35	565-575	5 cm
<i>Exp 4</i>	Natural with valves	32	600-645	—
	Artificial	39	320-330	3 cm

When the chest is opened the total ventilation is often increased, presumably because the distension of the lung is not curtailed by the chest wall. It does not follow, however, that because the lung is more inflated at each inspiration the blood is more adequately oxygenated, on the other hand, the fact that the lung is in the chest means that more

uniform inflation is likely to take place. Outside the chest the weaker portions tend to get most distended, whereas with the chest intact, this tendency is corrected by the lung pressing against the chest wall.

In our preliminary experiments in which the actual period of inspiration formed a much smaller proportion of the whole cycle and in which the pressure used in order to obtain the ventilation was greater, we often obtained such results, *i.e.* a conjunction of increased ventilation with decreased saturation of the arterial blood on opening the chest. In the above table the total ventilation during natural respiration is given as about 500–600 c.c. Such measurements are taken with Müller's valves and are probably in excess of the total ventilation of the normal cat when its respiration is unhampered.

The data given in Period I of the following sample experiment are probably more nearly normal. Here the total ventilation at the commencement of the experiment is only 250 c.c. per minute. The result is that the oxygen in the inspired air is only 15.56 p.c. and the CO<sub>2</sub> is 3.65 p.c. The oxygen in the inspired air differs from that of the atmosphere by over 5 p.c. These figures have three points in their favour:

(1) They are probably much nearer the normal ones than are the higher oxygen (18–19 p.c.) and lower carbonic acids (2–1 p.c.) in Periods II and III, which are obtained with ventilation of 500–800 c.c. per minute.

(2) As the basis of metabolism experiments, or experiments on the minute volume calculations, the smaller oxygen and carbonic acid differences given in the later periods entail a larger error.

(3) The animals probably become acapnic.

Hitherto I have not found it possible to maintain a long experiment with artificial respiration and the chest open with a low total ventilation and maintaining the oxygen in the expired air at about 16 p.c. and the carbonic acid at 3–4 p.c. To this extent our experiments have fallen short of the ideal.

<i>Exp. 5</i>	Period I natural respiration	Period II after 1 hour artificial respiration with chest closed	Period III commencing after operation for opening chest which takes about an hour	
			½ hour after chest open	3 hours after chest open
Body temperature	37° C.	37	36.7	36.5
O <sub>2</sub> consumption per min. in c.c.*	150	165	187	156
Total ventilation, dry air in c.c.	280	523	782	665
Arterial saturation with O <sub>2</sub> p.c.	95	95	95	92
O <sub>2</sub> in expired air, p.c.	15.65	18.31	18.62	18.78
CO <sub>2</sub> in expired air, p.c.	3.65	2.07	1.64	1.42
Respiratory quotient	0.68	0.79	0.70	0.65

\* Mean of two determinations.

*The oxygen in the arterial blood.* In Exp. 5 it will however be seen that with total ventilation of 500–800 c.c. per minute, the oxygen consumption of the cat was maintained to the end of the third hour after opening the chest, and the oxygen content of the arterial blood was also kept up to above 90 p.c. saturation.

On the whole, with the apparatus used, the oxygenation of the arterial blood has been, up to three hours, within the limits for normal cats, though in every case it has shown some slight drop. In one case within three hours of opening the chest the arterial saturation dropped to 88 p.c., a figure which would be low in man but which is not unfrequently met with in cats under operation. The general tendency is for a small drop to occur in the saturation at the end of three hours. Thus in five experiments the initial and final saturation may be compared.

Exp.	Initial saturation	Final saturation	Time which elapsed between initial and final saturations	Time of final saturation after opening chest	Emphysema
3	91	96	4 hours	3 hours	None
4	96	86	3½ "	2½ "	Slight
5	95	92	4 "	3 "	"
6	94	89	3½ "	1½ "	Edge only
7	98	90	3½ "	2½ "	Slight

In one experiment, however, the percentage saturation of the arterial blood fell to 82 p.c. In this case it is perhaps worth noting that even at the commencement of the experiment it was not very high (92 p.c.). In one experiment, No. 8, in which the emphysema was considerable, the final saturation was only 56 p.c.

#### SUMMARY.

The ordinary forms of artificial apparatus either do not oxygenate the blood completely when the chest is open or else produce emphysema when the experiment extends over a time measured in hours.

An apparatus is described by which artificial respiration can be maintained for nine hours or more with the chest open without producing emphysema, and which does not cause more than a trivial decrease in the oxygen saturation of the arterial blood.

I wish to express my hearty gratitude to Mr Barcroft for his advice and kind interest, which have enabled me to carry out this research, and to Prof. Langley for granting me the facilities of his laboratory.

## ON THE EFFECT OF ADRENALINE ON THE RESPIRATORY CENTRE. BY YAS KUNO.

*(From the Physiological Laboratory, Manchuria Medical College,  
Mukden.)*

THE inhibitory action of adrenaline on respiration was attributed by Boruttau in 1899(1) to a direct action on the respiratory centre, a view which has been supported by Nice, Rock and Courtright(2). On the other hand, Loewi and Meyer(3) and Roberts(4) have independently come to the conclusion that cessation of respiration caused by adrenaline is due to constriction of the cerebral blood vessels and consequent cessation of the blood supply to the respiratory centre. As the arguments in support of the vaso-constrictor theory seemed to me inconclusive, I made experiments in several ways, the results of which show, I think, that adrenaline apnoea cannot be due to cerebral vaso-constriction.

The experiments were all made on rabbits anaesthetised with urethane or ether.

1. In the first place I determined the effect of adrenaline on the rate of blood flow from the brain. A hole, about 4 mm. in diameter, was made in the occipital bone and the torcular Herophili. The openings of the lateral sinuses were closed with wax to prevent back flow from the general venous system.

A metal tube was inserted in the trephine opening, and this was directly connected with a water-manometer. When blood flows out slowly under normal conditions, the water in the manometer rises gradually. The changes of the manometer were recorded by means of a piston recorder, and the tracing indicates roughly the rate of outflow. The respiratory movements, the carotid pressure, and the pressure in the circle of Willis were simultaneously recorded. The pressure in the circle was obtained by inserting a cannula in the peripheral end of the common carotid, the external carotid of which was ligatured at its origin.

An illustration of the experiment is shown in Fig. 1, in which the respiratory movements were recorded by connecting one limb of the Y-shaped trachea tube to a rubber tambour. At the arrow 1 mg. of adrenaline was injected into the jugular vein. An apnoea was produced,

the arterial pressure rose, and a very marked increase of the venous outflow occurred a few seconds after the beginning of the rise of arterial

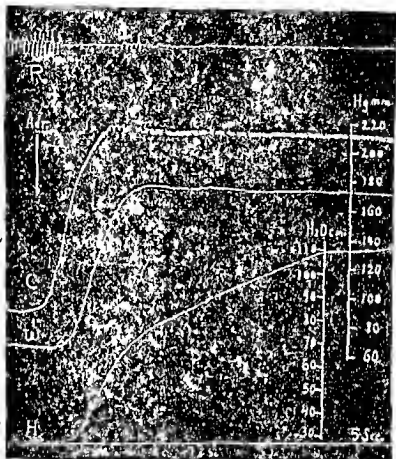


Fig. 1. *R*, respiration; *C*, carotid pressure; *W*, circle pressure; *H*, venous outflow.

pressure. The same experiment was successfully made on five animals; in all, the venous outflow was remarkably increased by adrenaline. The result shows that the cerebral circulation is augmented when apnoea is produced by adrenaline.

2. Roberts(4b) found that in the rabbit adrenaline caused a greater rise of blood-pressure in the circle of Willis than in the carotid and he concluded that the greater rise was due to contraction of cerebral blood vessels and consequent increased resistance. It can, however, be shown that there may be a similar difference in the degree of rise of blood-pressure in conditions in which there is no contraction of cerebral vessels. Thus, in one experiment in which the carotid pressure was 106 and the circle pressure 57 mm. Hg, I compressed the abdominal aorta; the carotid pressure rose 67 mm. Hg and the circle pressure rose 78.

Obviously the cause of changes of pressure in the circle can be more definitely determined if the rise of aortic pressure is reduced to a minimum. Accordingly I made eight experiments using the compensator described by Roberts.

In three out of eight experiments the circle pressure was very low from the beginning of the experiment and was about one-third of the carotid pressure. In these animals an injection of adrenaline caused a marked increase in the circle pressure which thereafter remained at the increased height. A second injection of adrenaline caused no rise but in most cases a slight decrease. In the remaining five experiments, the height of the circle pressure was about 65 to 75 p.c. of that of the carotid pressure. In these animals every injection of adrenaline caused more or less decrease in the circle pressure. One example of these experiments is illustrated in Fig. 2. The respiration was inhibited in all the cases,

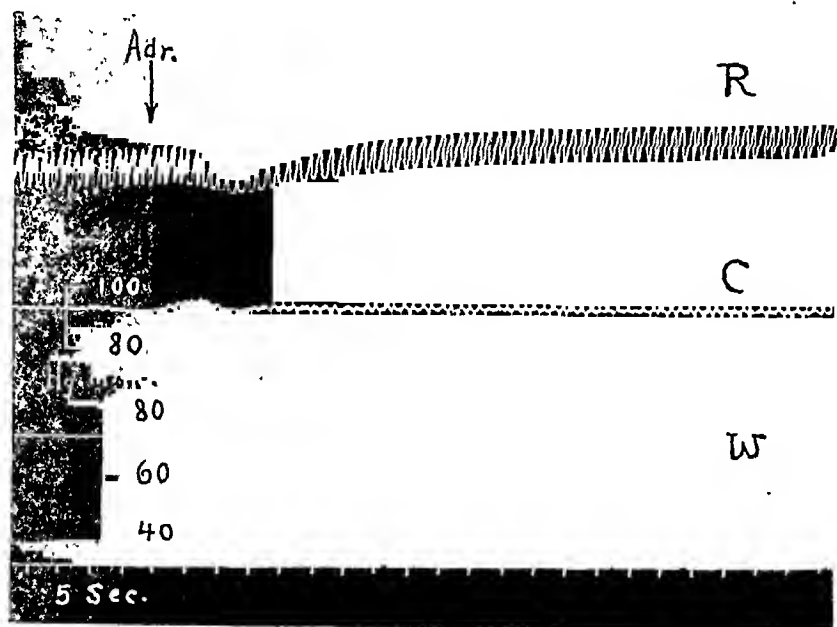


Fig. 2.

but the degree of inhibition was extremely slight (cp. Fig. 2) compared with that occurring when the compensator was not used. Whilst I have not definitely determined the cause of the discrepancy in the results, the explanation of them lies, I think, in the following facts. When the aortic pressure is kept constant the pressure in the circle of Willis depends partly on the resistance in the arteries between the aorta and the circle, which in relation to the circle we may call the central resistance, and partly on the resistance of the branches of these arteries (mainly of those of the external carotid), *i.e.* of the peripheral resistance.

Increase of central resistance will lower the circle pressure. Increase of peripheral resistance will raise the circle pressure. These changes occur independently of changes in the cerebral vessels. I suggest that the effect of adrenaline on the circle pressure depends on the balance of its effect on the central and peripheral resistance; that if the tone of peripheral vessels is low, the increase in peripheral resistance overcomes the effect of increase in central resistance, and that if the peripheral resistance is normal or high; the increase in central resistance is the more effective.

3. A weighty reason for considering that adrenaline apnoea is not due to cessation of circulation in the respiratory centre is that the phenomena accompanying it are very different from those accompanying cessation of circulation produced by arterial occlusion. Upon occlusion of the cerebral arteries the respiration, after a short interval, usually becomes faster and deeper, and then suddenly there are inspiratory spasms (or a single spasm) which lasts from  $\frac{1}{2}$  to 2 minutes. These then either gradually relax or are followed by a series of gasps. Thus the apnoea produced by occlusion of the arteries is due to a tetanic contraction of the muscles, whilst that produced by adrenaline is due to complete relaxation of the muscles. I may give some details of one experiment. This was made by Dr M. Kosaka of this Laboratory. He recorded the respiratory movements of the chest and abdomen simultaneously by means of stethographs. On the same animal, the apnoea was repeatedly induced by injection of adrenaline and by occlusion of the cerebral arteries. One pair of tracings obtained are given in Figs. 3 and 4. In Fig. 3 the arteries of the head were occluded at the arrow. In Fig. 4 adrenaline was injected. It will be seen that in Fig. 3 the apnoea was produced by an inspiratory tetanus, and that in Fig. 4 there was a gradual decrease of the respiratory movements. Roberts(5), in a paper published after my experiments were made, has also described and figured muscular spasms as occurring on occlusion of the cerebral arteries. His account is, however, different somewhat from that which I have given above, and he gives no explanation of the results. Roberts points out in this paper that a very small blood supply to the brain is sufficient to keep up respiration, and that respiration starts at once on releasing occluded cerebral arteries. These facts were also found in my experiments. They seem to me to afford an additional argument against the vaso-constrictor theory of adrenaline apnoea, for they show that on this theory adrenaline must cause complete, or nearly complete, occlusion of the cerebral vessels. If that were the case, the vaso-constriction ought to be readily detected in perfusion experiments and it is known that this is not the case.

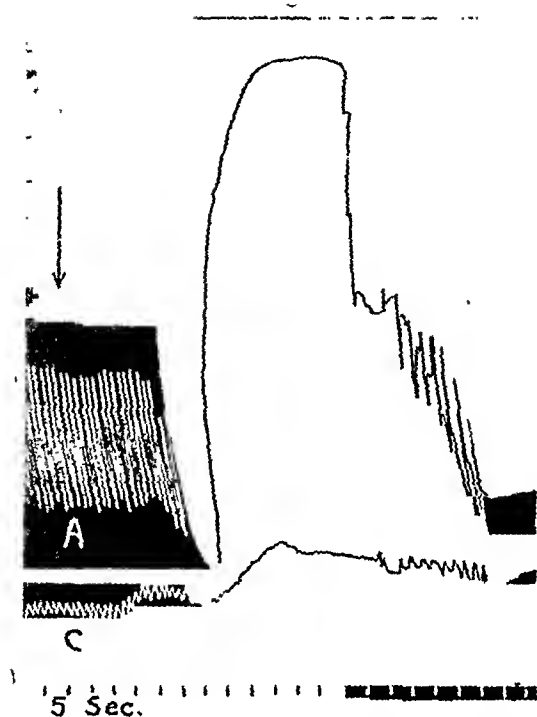


Fig. 3. Respiratory movements of abdomen (A) and chest (C). At the arrow the arteries of the head occluded.

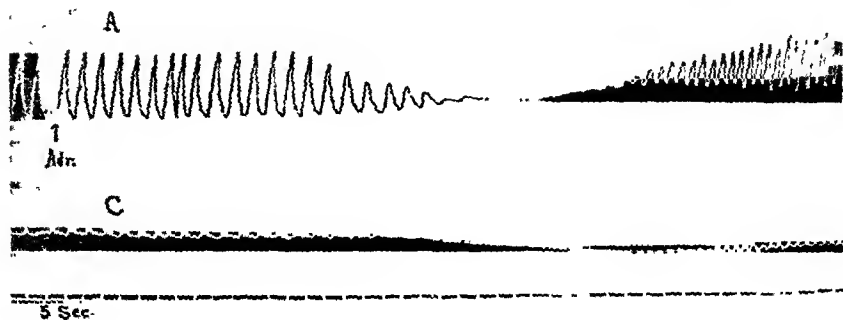


Fig. 4. Respiratory movements of abdomen (A) and chest (C). At the arrow 0.1 mg. adrenaline injected.



*Discussion.* The facts given above show, I think, that the apnoea produced by adrenaline is not due to its causing contraction of the cerebral blood vessels, and that the blood flow in the brain is increased and not decreased by adrenaline. It might be imagined that the increase in blood flow produced apnoea by washing out  $\text{CO}_2$ . Whilst this is probably a contributory action, it can hardly account for the whole of the apnoea, for we have seen that when the aortic pressure is kept practically constant by means of a compensator, some inhibition of respiration is still caused by adrenaline. On this account it must be concluded that adrenaline has some direct action on the nerve cells of the respiratory centre.

#### SUMMARY.

1. The rate of the venous outflow from the torcular Herophili is greatly increased by an intravenous injection of adrenaline during the apnoea produced by the injection.

2. When the general arterial blood-pressure is kept practically constant by a compensator, and the blood-pressure in the circle of Willis is fairly high, injection of adrenaline causes a slight fall of pressure in the circle and incomplete inhibition of respiration (5 experiments). If, however, the pressure in the circle is low, adrenaline causes a slight rise of blood-pressure in the circle (3 experiments). The different effects on the circle blood-pressure are considered to be due to a difference in the relative contraction of the arteries running to the circle and of the peripheral branches outside the brain.

3. Occlusion of the cerebral arteries causes apnoea by producing tetanic contraction of the respiratory muscles. Adrenaline, as known, produces apnoea by causing complete cessation of respiratory movement.

4. It is concluded that adrenaline does not cause apnoea by constricting the cerebral vessels, but partly by increasing the cerebral blood flow and washing out  $\text{CO}_2$ , and partly by a direct action on the nerve cells of the respiratory centre.

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## NOTE.

Subsequent to my writing the account of my experiments, three papers have appeared referring to the effect of adrenaline on cerebral vessels. Huggett and Mellanby (*This Journ.* 59, p. 387, 1924) withdraw the support they had previously given to the theory that adrenaline apnoea is caused by cerebral vaso-constriction. Their arguments have been contested by Roberts (*Ibid.* 59, p. 460, 1925). Anrep and Starling (*Proc. Roy. Soc. B*, 97, p. 463, 1925) have shown that when the driving force of an artificial circulation through the head is maintained constant, injection of adrenaline causes a rise of circle pressure (in the dog) in consequence of constriction of all the branches of the carotid which supply the skin and muscles of the head and neck.

## FURTHER OBSERVATIONS ON THE PRODUCTION OF ANHYDRÆMIA WITH INSULIN.

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New Haven, Connecticut.)*

Dogs, given large doses of insulin (20 units per kilogram of a standardised extract furnished by Eli Lilly and Co.), have been found by the writer and Edwards(1) to develop evidences of anhydræmia. This condition was postulated upon the basis of our finding a significant, rapid increment in the percentage of hæmoglobin (14.5-44.2 p.c.), a great increase in the cell count, a decrease in the total blood volume, a reduction in the percentage of plasma, and also physical evidence of blood dehydration—increased viscosity and apparent anoxæmia. The results were the same in unanæsthetised animals as in those narcotised with iso-amyl-ethyl-barbituric acid (Amytal), which *per se* has no effect on the content of blood sugar(2), blood-pressure(2), and blood concentration(1).

Very similar results have been obtained by Olmsted and Taylor(3), who administered insulin to decerebrated and decapitated cats. These observers found that the operative procedures alone caused no blood concentration. Villa(4) has found that the administration of 40 to 50 units of insulin to diabetic and non-diabetic persons produces a decrease in blood volume and a rise in the refractive index of the serum. The most recent corroboration of our results came from the Toronto laboratories in a paper by Best and Ridout(5). There is evidence, however, that the restoration of a hyperglycæmia to normal does not have the same influence upon blood concentration as does the production of severe hypoglycæmia with large amounts of insulin. In this connection the work of Banting and Best(6), Staub, Gunther and Fröhlich(7), and Widal, Abrami, Weill and Laudat(8) may be cited.

That the rabbit apparently reacts differently from the carnivora was originally pointed out by Olmsted and Taylor(3). They found the blood reaction of this species to insulin to be quite inconstant. Most of their animals showed a slight blood dilution when the blood was drawn from the larger veins although there was an apparent blood concentration

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peripherally, often making it impossible to draw blood from the ear vein. Blood dilution, in the rabbit, was also found by Haldane, Kay and Smith<sup>(9)</sup>. A later report by Kay and Smith<sup>(10)</sup>, however, stated that in the rabbit blood dilution is not a "specific" insulin effect. A number of their animals developed, rather, a concentration of the blood in the early stages of the reaction. On the other hand, a recent report by Levine and Kolars<sup>(11)</sup> indicated that rabbits, too, may regularly develop anhydræmia after the administration of insulin.

Hamilton, Barbour and Warner<sup>(12)</sup>, using a new method for specific gravity estimation<sup>(13)</sup>, have failed to demonstrate an increased specific gravity of the blood in four dogs to which they administered "convulsive" doses of insulin. Upon this basis, although two of the animals showed a temporary rise in dry blood solids, these investigators have concluded that there is no blood concentration attending the hypoglycæmia induced by insulin.

In view of the contradictory findings of the several observers, the writer has deemed it advisable to carry out further experiments on dogs and rabbits to determine the condition of the blood in insulin hypoglycæmia.

*Methods.* No anæsthetic was employed. Blood samples were drawn mainly from the heart and the jugular in the case of the dogs, from the heart and the ear vein in the rabbits. After taking a control sample in each case, a large dose of insulin (20 units per kgm. body weight) was administered subcutaneously or intravenously. One hour following the injection, a second blood sample was drawn and further samples at approximately half-hour intervals. The blood sugar was determined by the method of Shaffer and Hartmann<sup>(14)</sup> and the hæmoglobin, as an index of blood concentration, by a modification of the Cohen and Smith method<sup>(15)</sup>. In five out of the seven dogs and in two of the three rabbit experiments a check upon the hæmoglobin was made by determining the dry solids of the blood before and after administering insulin. In one case the changes in the relative density of the blood were determined gravimetrically. As in the previous work, visible changes in certain physical properties of the blood, such as viscosity, tendency to clot and changes in colour were noted.

*Effect of insulin on the blood of dogs.* Following the administration of the insulin there was a rapid drop in the blood sugar level. The blood was virtually "purged" of its sugar, as determined by the method of Shaffer and Hartmann, falling from normal (140 to 88 mgm. per 100 c.c.) to 30-0 mgm. per 100 c.c., in the first hour. Accompanying

TABLE I

Exp	Vessel tapped	Blood sugar		Hæmo-globin		Dry blood solids		Len. of tube after insulin	Increment		Blood concentration	
		Before insulin	After insulin	Before insulin	After insulin	Before insulin	After insulin		in hæmo-globin	Increment blood solids	calculated from hæmo-globin increment	calculated from blood solids increment
Dogs								hr min	p c	p c	p c	p c
1	Heart	140	022	106.3	146.7	21.8	25.7	3 2	40.4	3.9	38.0	18.2
2	Heart	122	012	99.8	124.3	22.0	24.9	2 55	24.5	2.9	24.6	13.3
3	Ext jugular	088	000	100.4	117.2	18.5	20.3	2 30	16.8	1.8	16.7	9.7
4*	Heart	106	040	115.9	190.4	21.2	28.3	3 1	74.5	7.1	64.3	33.5
5	Ext jugular	099	013	108.2	138.5	14.3†	1.73†	2 10	30.1	—	28.0	—
6	Heart	091	028	103.5	131.6	—	—	2 22	28.1	—	27.1	—
7	Heart	093	026	115.9	145.3	—	—	3 9	29.4	—	25.4	—
8	Ext jugular	104	030	112.7	152.5	22.9	26.3	2 30	39.8	3.4	35.3	14.8

\* Same animal as 3, but thirsted (water starved) for 5 days before experiment

† Relative densities of blood, determined gravimetrically. Calculated from these values blood concentration is 20.9 p c

TABLE II

Rabbits		p c	p c	p c	p c	p c	p c	hr min	p c	p c	p c	p c
1	Heart	180	070	92.3	98.0	19.4	19.5	1	0	5.7	0.1	6.2
2	Int vein	170	082	100.2	108.0	18.6	19.8	58	7.8	1.0	7.7	6.4*
	Heart	175	063	100.0	84.0	18.7	17.9	59	-16.0	-0.8	-16.0	-4.2
3	Heart	174	078	97.6	100.3	—	—	1	3	2.7	—	—

\* Sudden death      Autopsy      sum. amount pericardial effusion

† Sudden death      Autopsy      negative

\* Sudden death Autopsy sum. amount pericardial effusion

† Sudden death Autopsy negative

the fall in blood sugar there was found in all cases a marked concentration of the blood (16.7–38.0 p.c. in this series), as determined by the rise in the percentage of hæmoglobin. The values for dry blood solids showed corresponding though smaller increases (9.7–18.2 p.c.). In one experiment, in which the relative density of the blood before and after insulin administration was determined gravimetrically, the values were 1.43 and 1.73 respectively. The appended table presents the essential results of the several experiments. There is included one experiment (No. 4) of another series which has recently been reported<sup>(10)</sup>. The extreme anhydræmia of this dog is characteristic of animals which have been thirsted for a long time and then given insulin.

Once again, as in the previous experiments, the physical qualities of the drawn blood samples were striking. After use of the insulin, the blood of the dog drawn from the jugular or from the heart was more viscid, clotted more rapidly and was distinctly darker (apparently anoxæmic). The magnitude of these changes was always greatest in the samples highest in hæmoglobin and seemed roughly proportional to the degree of blood concentration. It is of interest to note also that, in those cases in which convulsions occurred, the concentration of the blood was in evidence long before the muscular phenomena.

*Effect of insulin on the blood of rabbits.* Hyper-excitability and fright during the period of the experiment were the rule. There was a slight, primary hyperglycæmia (170–180 mgm. glucose per 100 c.c.). The sugar did not fall to the low level observed in the dogs but the reduction was definite (the lowest value having been 63 mgm. per 100 c.c.). The blood of one of the animals taken from the heart showed a slight concentration (6.2 p.c.). In another animal, the blood from the heart showed a 16 p.c. dilution and that drawn simultaneously from the ear vein a concentration of 7.7 p.c. The finding of dilution centrally may have been due to a pericardial effusion found at autopsy. The possibility of local, peripheral stasis is also to be considered. The dry solids exhibited proportional changes. The second table shows the more detailed results.

*Discussion.* These experiments have entirely justified our former conclusion<sup>(1)</sup> that large doses of insulin produce an anhydræmia in dogs. The absence of concentration in the few experiments of Hamilton, Barbour and Warner<sup>(12)</sup> may have been due to the use of too small amounts of insulin. This is suggested by the low magnitude of the blood sugar changes and the unusual report of these investigators that two of their animals died with a hyperglycæmia after the resuscitative administration of glucose. Experience has taught the use of "mass" doses of

insulin (20 units per kgm. body weight) in this type of experimentation. Such a dose insured the production of a rapid, profound hypoglycæmia in all dogs. Rapid changes were here essential(1, 17, 18). The administration of 150 to 200 c.c. of 10 p.e. glucose has always insured a remarkably rapid recovery of animals that have received this large amount of insulin.

The results in the rabbits were inconstant. These animals, unanæsthetised, seemed to develop severe "tachycardial" attacks when merely touched on the abdomen after resting for an hour. The nervous condition of the rabbit was deemed by the writer to render this species unfit for this type of blood study. This irregularity of response was confirmatory of the work of Olmsted and Taylor(3) and explained the antithetical results of Haldane, Kay and Smith(9) on the one hand, and of Levine and Kolars(11) on the other.

No anæsthetic was used in this series of experiments and the concentration of the blood was, therefore, not the result of the combined action of two drugs as has been suggested(9, 12). The possibility that protein impurities may be the cause of the decrease in blood volume is unlikely. The preparation of insulin was a standardised, purified product and the writer has never observed any symptoms suggestive of anaphylaxis in animals given repeated doses of this extract. Haldane, Kay and Smith(9) have suggested that our results(1) might be accounted for by the presence of a "pressor" substance in our insulin preparation. It seems to the writer that the dehydration of the blood as the direct result of the sudden, profound hypoglycæmia is a far more tenable hypothesis. Such a view is consistent with known physiological phenomena. Lond on and Polowzowa(10) have shown that the introduction of a concentrated solution of sugar into the small intestine causes water to be drawn into the intestinal lumen, the height of absorption not being reached till the concentration of the sugar has been reduced to about 6 or 8 p.c. Omi(20) has extended these results by finding that the resorption of glucose from the intestine reaches its maximum at a concentration corresponding with the osmotic pressure of the blood serum. The additional observation that the absorption of glucose results in a temporary hyperglycæmia which is shortly followed by an attraction of water into the blood—blood dilution—was the contribution of Fisher and Wishart(21). Our work apparently has demonstrated the antithesis of the condition of hyperglycæmia and hydræmic plethora, namely, hypoglycæmia and anhydræmia. That the loss of water from the blood is related to the withdrawal of blood sugar rather than to a "specific" insulin effect is further

emphasised by the work of Underhill and Karelitz<sup>(22)</sup> on hydrazine poisoning. In this condition, too, hypoglycæmia is associated with a concentration of the blood.

### SUMMARY.

The earlier work of Drabkin and Edwards<sup>(1)</sup> was confirmed. After large doses of insulin, associated with a rapid, pronounced hypoglycæmia, dogs invariably developed anhydræmia.

Unanæsthetised rabbits were irregular in their response and were found to be ill-adapted for such a study.

It has been again pointed out that the use of "mass" doses of insulin is essential in this type of experiment.

The sudden, profound hypoglycæmia—the virtual purging of the blood of its sugar—is thought to be the primary cause of the anhydræmia.

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ON HÆM IN NATURE. BY M. L. ANSON  
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(*From the Physiological Laboratory, Cambridge.*)

I. THE CONSTANCY AND THE DISTRIBUTION OF HÆM.

It was believed, even fifty years ago, that the various hæmoglobins from different animals were different. But much of the evidence for this belief was of questionable value. Sorby<sup>(1)</sup>, however, showed definitely in 1876 that the spectrum of *Planorbis* hæmoglobin was different from that of human hæmoglobin. More recent work has proved not only that the spectrum of hæmoglobin varies from species to species, and sometimes even within the same species, but also that the affinity for oxygen varies from hæmoglobin to hæmoglobin. Sorby himself first suggested, though without any experimental evidence, that this hæmoglobin specificity was a *protein* specificity, and that the iron-pyrrol component of hæmoglobin, hæm, was invariable.

Hæmoglobin is found in the bloods of all vertebrates. This is about the only generalisation that can be made about its distribution. Its occurrence is very widespread throughout the animal kingdom, but it is absolutely haphazard, not following at all the usual evolutionary classifications. Hæmoglobin has been found in the starfish, *Ophiactis virens*, and in no related forms. It has been reported in the body fluids of the larvæ of two or three insects, although not in any other insect larvæ or in the body fluids of any adult insects<sup>1</sup>. All the pulmonate snails, with one exception, contain hæmocyanin. That one exception, *Planorbis*, contains hæmoglobin. Some worms have hæmoglobin, some chlorocruorin, some neither pigment. A hæmoglobin occurs occasionally, though on the whole very rarely, in muscle, for instance in the striped and heart muscle of many vertebrates, and in the pharyngeal muscles of certain gastropods. Frog muscle contains none. In the parasitic worm, *Ascaris*, not only the muscles but also all the tissues except those of the generative organs are impregnated with hæmoglobin. Apparently a very complicated substance with very peculiar properties has been evolved independently again and again in nature.

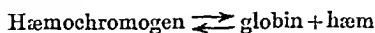
<sup>1</sup> The ancient and often-repeated statement that the blow-fly *Musca* contains hæmoglobin has no basis in fact.

A number of natural pigments have been described which are related to hæmoglobin in that they are either themselves hæmochromogens or can easily be converted into hæmochromogens by the action of alkali and a reducer. Their occurrence is again haphazard and their functions for the most part are unknown. Moreover, the hæmochromogens from these pigments vary definitely amongst themselves, and they are all different from the hæmochromogen made from hæmoglobin. The current notion is that hæmochromogen is the iron-pyrrol component of hæmoglobin, that it is merely the reduced form of hæm. On the basis of this notion one would have to conclude that corresponding to the different natural hæmochromogens there are different hæms.

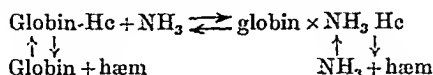
Hæmochromogen, however, is *not* merely the reduced form of hæm. We have recently demonstrated<sup>(2)</sup> that every hæmochromogen consists of hæm chemically combined with a nitrogen substance and that in particular the hæmochromogen made from hæmoglobin is itself a compound of hæm and globin. So not only is it conceivable that *all* the hæms in nature are identical, being merely attached to different nitrogen compounds, but the hæmochromogens even from the different hæmoglobins must be different, for they all contain different globins.

The simplest and the most sensitive method for investigating the question of the identity of the hæms is unquestionably the spectroscopic. Hæm itself has not a very definitely defined spectrum. The main band of hæmochromogen, in contrast, is very sharp, and the position of its maximum can be determined with the Hartridge reversion spectroscope to within 1 Å unit. It is desirable, therefore, rather than to compare the hæms themselves, to convert them into hæmochromogens containing the same nitrogen compound and to compare the resulting hæmochromogens.

Globin-hæmochromogen is slightly dissociated into its components, globin and hæm<sup>(2)</sup>.



If a nitrogen substance, *e.g.*  $\text{NH}_3$ , which also combines with hæm, is added to the system the following equilibrium is obtained:



The exact position of the equilibrium depends on the relative affinities of globin and  $\text{NH}_3$  for hæm and on the relative concentrations of globin and  $\text{NH}_3$ . If globin had a thousand times greater affinity for hæm than  $\text{NH}_3$  but  $\text{NH}_3$  in compensation were present in a thousand times greater

concentration than globin, then at equilibrium there would be equal concentrations of the two forms of hæmochromogen. It so happens that the affinity of globin for hæm is so much greater than that of  $\text{NH}_3$  that several hundred c c of concentrated  $\text{NH}_3$  have to be added to the globin-hæmochromogen corresponding to 1 c c of blood in order to shift the equilibrium represented above to the right. The  $\alpha$  band of  $\text{NH}_3$ -hæmochromogen is 20 Å to the blue of the  $\alpha$  band of globin hæmochromogen. As one adds more and more  $\text{NH}_3$  to globin-hæmochromogen, one gets mixtures containing relatively more and more  $\text{NH}_3$ -hæmochromogen, and the band given by the mixture which results from the fusion of two bands of the two hæmochromogens is gradually shifted to the blue. Once the band reaches the position characteristic of  $\text{NH}_3$  hæmochromogen the addition of more  $\text{NH}_3$  has no further influence on its position.

Theoretically the above procedure can be used whatever the affinity of the natural nitrogen compound for hæm. The greater the affinity the more  $\text{NH}_3$  one has to add in order to shift the equilibrium to  $\text{NH}_3$ -hæmochromogen. Practically, however, the hæmochromogen must be present in a certain concentration if the spectrum is to be measured and so a limit is set to the degree which the solution can be diluted with  $\text{NH}_3$ . In cases where the affinity of the nitrogen compound for hæm is much greater than that of globin it is necessary to separate the hæm from the nitrogen compound by Schulz's (3) method and to add the  $\text{NH}_3$  to the pure hæm.

The Schulz separation depends on the fact that acid hæmatin is slightly dissociated into its components, a nitrogen compound which is soluble in acid and hæm which is practically insoluble in acid (2). If ether is added the hæm passes over into the acid ether in which it is very soluble. More hæmatin then has to dissociate to maintain the equilibrium. Thus, eventually, all the hæmatin dissociates, all the hæm goes over into the ether phase and all the nitrogen compound remains in the aqueous phase. The hæm is easily extracted from the ether with alkali.

Pyridine can be used instead of  $\text{NH}_3$ . It has a very great affinity for hæm, much greater than that of  $\text{NH}_3$ . And it is so efficient a solvent that we are now using it for the extraction and estimation of tissue hæm. There is one complication. Pyridine can influence the spectrum of hæmochromogen not only by acting as a nitrogen compound but also by acting as a solvent, for the spectrum of a pigment is by no means independent of the solvent. If pyridine is added to the fluid, which contains hehcorubin, a natural hæmochromogen, the fluid would dilute the pyridine and a pyridine hæmochromogen might possibly be

formed whose spectrum were slightly different from that obtained when hæm is dissolved in *pure* pyridine. This complication may not be serious if the conditions are properly chosen. In any case, it is advisable to avoid it altogether by using the  $\text{NH}_3$  method when dealing with solutions.

It has often been stated—usually without any experimental proof whatsoever—that the hæms from all the different hæmoglobins are all the same. The proof by elementary analysis of the identity of the hæms is not conclusive, for elementary analysis cannot always distinguish between hæmoglobins which are known to be different. Hæmin crystals are supposed to be the same whatever the blood from which the hæmin is made. We have found (4) the spectra of hæmochromogens prepared by alkaline “hydrolysis” of different hæmoglobins to be the same. That is, although the different globins cause differences in the hæmoglobin spectra which are easily measured, the differences caused by these same globins in the hæmochromogens are too small to be detected with the Hartridge spectroscope. The spectroscopic method, sensitive though it is, has its limits. It is conceivable, of course, that there are small differences in the hæms themselves, which are not brought to light by the spectroscopic measurements. It is, however, highly improbable. One can synthesise human hæmoglobin with its characteristic spectrum from human globin and the hæm from another species, and  $\text{NH}_3$ - and pyridine-hæmochromogens are the same whatever hæmoglobin is the source of the hæm. All the experimental data then go to support the hypothesis that in the hæmoglobins the hæm part is invariable.

MacMunn (5) has given the name actinohæmatin to a pigment found in many actiniæ. He discovered that this pigment is converted into hæmochromogen by the action of alkali and a reducer. We used as a source of actinohæmatin *Actinea equina*. The whole animal was ground in a mortar and the ground-up tissue was left over night in concentrated  $\text{NH}_3$ . The  $\text{NH}_3$  extract was found to contain a hæmochromogen whose main band was in exactly the same position as that of  $\text{NH}_3$ -hæmochromogen prepared from hæmin and  $\text{NH}_3$ . Actinohæmatin hæm is therefore the same as hæmoglobin hæm.

Helicorubin (6) is a hæmochromogen found in the liver and gut of certain snails. The main band of the alkaline form is some 35 Å to the red of the main band of  $\text{NH}_3$ -hæmochromogen. The affinity of the nitrogen compound of helicorubin for hæm is so great that this band cannot be shifted much by the addition of any practicable amount of  $\text{NH}_3$ . If  $\text{NH}_3$  is added to the hæm after its separation from the nitrogen compound by Schulz's method, then the typical  $\text{NH}_3$ -hæmochromogen

is obtained Helicorubin hæm is therefore the same as hæmoglohu hæm

The same hæm, then, is found here and there in the animal kingdom in a variety of pigments It seems at first sight as if hæm had repeatedly been developed independently in nature An entirely new light, however, is brought to the question by the recent work of Keilin (7) on the respiratory catalyst cytochrome Cytochrome seems to be present in all animal cells and it is easily detected in yeast and in many other plant cells and in bacteria The pigment itself has a complicated spectrum On the addition of alkali there appears a typical hæmochromogen This hæmochromogen can combine loosely with CO—a reaction characteristic of hæmochromogens Keilin further discovered that many reserve tissues, particularly in plants, contain a pigment different from cytochrome which even in nature has the typical hæmochromogen spectrum We have extracted with pyridine on the one hand bee's muscle and yeast, which contain a great deal of cytochrome, and on the other hand, the fat body of the larva of the meat fly (*Calphora*), the bean of the scarlet runner, and the shallot (a relative of the onion), all of which contain the hæmochromogen referred to In all cases we obtained a pyridine hæmochromogen whose  $\alpha$  band was in exactly the same position as that of the pyridine hæmochromogen made from the hæm of sheep hæmoglobin Hæm is *universal*

Pyridine hæmochromogen can be characterised not only by its spectrum but by certain peculiar properties First, when dissolved in pyridine it remains reduced even when exposed to pure oxygen Globin hæmochromogen, in contrast, when exposed to the air, is converted into hæmatin, its oxide Secondly, if the pyridine hæmochromogen is made acid, it combines with the oxygen of the air to form not hæmatin, which is analogous to methæmoglobin, but an oxy compound analogous to oxyhæmoglobin from which the oxygen can easily be pumped off It is worth noting that the influence of pH on the affinity of pyridine hæmochromogen for oxygen is just the opposite of the influence of pH on the affinity of hæmoglohu for oxygen The pyridine hæmochromogens prepared from the various natural hæms all possess, in addition to the typical pyridine hæmochromogen spectrum, these peculiar properties

We still do not know why some animals contain actinohæmatin or helicorubin or hæmoglobin and others do not Nor have we any notion of the mechanism by which the proper nitrogen compound is produced What is clear now is the *possibility* on the basis of a single hæm of a variety of hæm pigments having very different properties in adaptation

to very different needs and of their haphazard distribution. For hæm is universal, the nitrogen compound can change radically the properties of hæm in one direction or another, and given hæm, a suitable nitrogen compound, and physiological conditions, the synthesis of a hæmochromogen or a hæmoglobin is automatic(2). It is no longer necessary to assume a "special creation" for each hæmoglobin or hæmochromogen; they can be regarded merely as specialisations of a substance—hæm—existing in all cells.

There arises from this investigation the question of how much of the tissue iron is in the form of hæm. We shall report in another paper the results of analyses undertaken to answer this question.

## II. EQUILIBRIA INVOLVING HÆM *IN VIVO* AND *IN VITRO*.

The pyridine extract of yeast shows a three-banded spectrum (see Fig. 1). Band *A* corresponds to the band in the red of cytochrome shifted

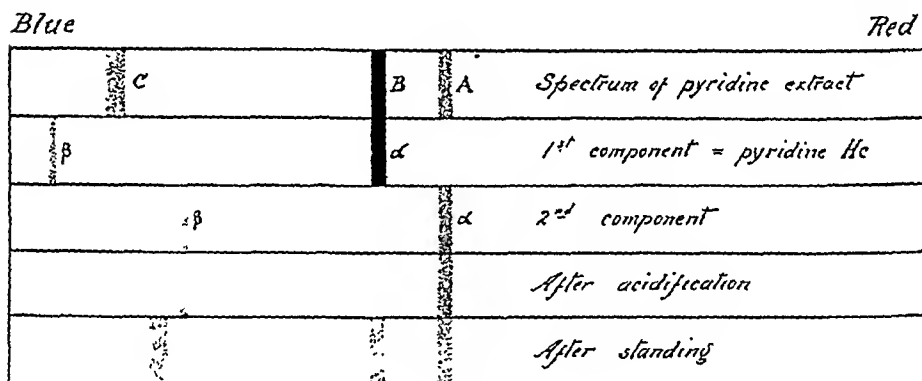


Fig. 1.

some 200 Å to the blue. Band *B* corresponds exactly to the  $\alpha$  band of ordinary pyridine-hæmochromogen from hæmin. And band *C* is somewhat to the red of the  $\beta$  band of pyridine-hæmochromogen. This three-banded spectrum, it seems, results from the mixture of two two-banded spectra (see Fig. 1), one caused by ordinary pyridine-hæmochromogen, the other by a compound which if it is a hæmochromogen contains a different hæm. Band *C* would thus be the result of the fusion of the  $\beta$  bands of these two components. The evidence that the pyridine extract of yeast really contains two different components is, firstly, that one component can be oxidised while the other remains reduced and, secondly, that the ratio in which the two components are present can be varied.

As has already been stated, if ordinary pyridine hæmochromogen is made acid in the presence of air it goes over into the oxy form. The spectrum of the oxy form is so much fainter than that of the reduced form that it cannot be detected in the usual pyridine extract of yeast, which shows the reduced form easily. The second component of the pyridine extract is unaffected or affected but little by this treatment with acid. It remains reduced. Thus, as a net result (see Fig. 1) band *A*, which belongs to the second component alone, remains unchanged in position and there is no great change in intensity, band *B*, which belongs to pyridine hæmochromogen alone, disappears entirely and band *C*, which was a mixture of the original  $\beta$  bands, becomes fainter and is shifted to the red. In other words, one component of the pyridine extract can be oxygenated independently of the other and thus the spectrum of one component is removed and one can see what is practically the spectrum of the other components in the reduced form. In the living yeast all the components of cytochrome seem to be oxidised and reduced simultaneously.

If a pyridine extract is let stand the spectrum fades gradually but not evenly (see Fig. 1). Band *B*, which originally was much more intense than band *A*, finally becomes less intense than band *A*. That is, the pyridine-hæmochromogen component has been fading more rapidly than the other. Corresponding to this and predictable from it is a shift (actually about 15 Å) of band *C* towards the red, towards the  $\beta$  band of the component which after the fading is present in *relatively* greater concentration.

It is definitely established, then, that the pyridine extract, and hence cytochrome, has at least two components, one of which contains a hæm which is identical with the hæm of hæmoglobin.

The spectrum of cytochrome, according to Keilin, has six bands and can be analysed into three two-banded spectra of three hæmochromogen-like components (see Fig. 2). Let us leave out of consideration the band in the red, and its corresponding  $\beta$  band  $\beta_1$ . The two hæmo-

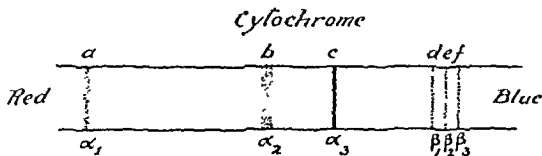
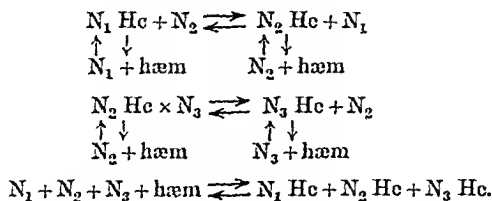


Fig. 2.

chromogens responsible for the remaining four bands are different, for their spectra are different. If pyridine is added to yeast these two hæmochromogens disappear and are both replaced by a single third hæmochromogen, namely pyridine-hæmochromogen. Since every hæmochromogen consists of two parts, a hæm part and a non-hæm nitrogen containing part, this pyridine experiment means that the two different hæmochromogens of cytochrome both contain the same hæm part, that in cytochrome this hæm is attached to two different nitrogen compounds, and that both these nitrogen compounds are replaced by a single third nitrogen compound, pyridine.

We must now face the fact that cytochrome is not a unit of fixed and constant composition; the proportion in which the various components are present varies from tissue to tissue. The evidence for this is that the *relative intensities* of the bands vary. To quote from Keilin's observations, in the muscle of the bee band *C* is very much more intense than band *B*, in yeast band *C* is more intense than band *B*, but the difference is much less than in bee muscle; in the pharyngeal bulb of *Helix aspersa* the intensity of the two bands is about equal, and, finally, in the cytochrome of the chitin of the blow-fly, the situation is actually reversed, and band *C* is less intense than band *B*.

Cytochrome, then, is a variable mixture of one sort or another. To understand the nature of this mixture we must remember that every hæmochromogen is dissociated into its two constituents, a hæm and a nitrogen compound, and that these two constituents are in dynamic equilibrium with the undissociated hæmochromogen. If more nitrogen compounds are added to the system a complicated equilibrium results in which all the nitrogen compounds compete for the hæm.



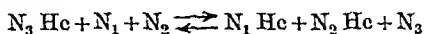
How much of the various hæmochromogens will be found depends on the relative concentrations of the nitrogen compounds and on their relative affinities for hæm. A simple example of such a system has already been described in detail, namely, hæm plus globin plus ammonia. Given hæm in the living yeast cell, then, there must be a complicated equilibrium, in which a great variety of nitrogen compounds compete for



the hæm. Any change in conditions which results in a change in the nature or concentration of these compounds or in their relative affinities for hæm will also result in a change in the hæmochromogen equilibria. One point needs emphasis here. Although many natural nitrogen compounds have a small affinity for hæm, the possession of a very great affinity for hæm, such as that of globin or the nitrogen compound of helicorubin, is a particular and highly specialised property. For instance  $X$  molecules of hæm, plus the corresponding amount of globin, will give practically  $X$  molecules of undissociated hæmochromogen. To get the same yield of undissociated hæmochromogen from the same  $X$  molecules of hæm, starting with egg albumin, serum globulin, casein or gelatine, none of which have so great affinity for hæm as globin, one would have to use large excesses of these proteins. Thus, while theoretically the hæmochromogen equilibria are almost infinitely complex, practically only a small number of highly specialised nitrogen compounds are involved. Given, then, in the cell, individual hæm molecules, several different kinds of nitrogen compounds competing for these hæms, and variations in conditions which favour one or another of these competing nitrogen compounds, there would result what one actually observes, a spectrum composed of several different hæmochromogen spectra and variations in the relative intensities of the bands. These equilibria with the nitrogen compounds might possibly exist even were several individual hæms united in cytochrome. It is improbable, however, that such a union would be compatible with the hæmochromogen type of spectrum.

Keilin has made the remarkable observation that the typical hæmochromogen found in certain plant reserve tissues can easily be converted into typical cytochrome. This can be done, for instance, without the addition of any reagent, simply by drying. A single band of the reserve tissue hæmochromogen clearly splits into bands  $B$  and  $C$  of cytochrome. We have already demonstrated that bands  $B$  and  $C$  are due to two hæmochromogens, both of which in common with the reserve tissue hæmochromogen, have the same hæm as hæmoglobin. This constancy of the hæm is what makes the transformation under discussion at all possible. But since the hæm does not vary, and the hæmochromogens do, it follows that the non-hæm parts of the hæmochromogens must vary. The two nitrogen compounds of the cytochrome hæmochromogen ( $N_1$  and  $N_2$ ) must be replaced in the reserve tissue hæmochromogen by a different nitrogen compound  $N_3$  (or perhaps by  $N_3$  and  $N_4$ , the  $\alpha$  bands of the two hæmochromogens fusing). The drying in some way has increased either the concentrations of  $N_1$  and  $N_2$  relative to the con-

centration of  $N_3$  or in their relative affinities for hæm. And this, in turn, has shifted the equilibrium



to the right. As the drying proceeds one can easily follow this gradual shift spectroscopically. Nothing is known about the nature of these various nitrogen compounds.  $N_3$  may be some simple tautomeric modification of  $N_1$  or  $N_2$ , may be entirely unrelated to  $N_1$  or  $N_2$ , or some of the hæmochromogens may be related to each other as are  $\alpha$  and  $\beta$  globin hæmochromogens.

Thus we see in nature variations in the hæmochromogen equilibria, some of which involve changes in the proportions of the nitrogen compounds attached to hæm, which are reflected by changes in the relative *intensities* of the bands, while others involve the actual substitution of different nitrogen compounds which is reflected in changes in the *positions* of the bands.

This leads us to a consideration of the biological significance of the variations in the hæmochromogen equilibria. We have shown(2) that though all hæmochromogens have certain properties in common, these properties can be changed slightly or greatly by suitable changes in the nitrogen compound. The various hæmochromogens involved in the equilibrium system called cytochrome must thus have different chemical properties, and any changes in the proportions in which they are present must result in changes in the properties of that system. On the other hand, respiratory requirements vary from cell to cell. They are not the same in the muscle of the meat fly larva, which, like all muscles, contains cytochrome as in the fat body of that larva which contains, like many plant reserve tissues, the reserve tissue hæmochromogen. They are not the same in the muscle of a bee where band *C* is much more intense than band *B* as in the chitin of the blow-fly, where band *C* is less intense than band *B*. It is reasonable to put these two sets of facts together and to consider the variations in the hæmochromogen equilibria as adaptations to variations in respiratory needs. A neater or more finely adjustable mechanism could hardly be imagined.

The reality and nature of the equilibria in question are further illustrated by the following experiments. When yeast is extracted with NaOH one obtains a three-banded spectrum, one band of which is pictured diagrammatically in Fig. 3. (We have omitted a band in the yellow-red which is irrelevant to the present discussion, and a band in the green, which could not be measured accurately.) The band pictured is broad,

fuzzy and uneven. The part of the band towards the blue is much darker than the part towards the red. If several successive extractions of yeast

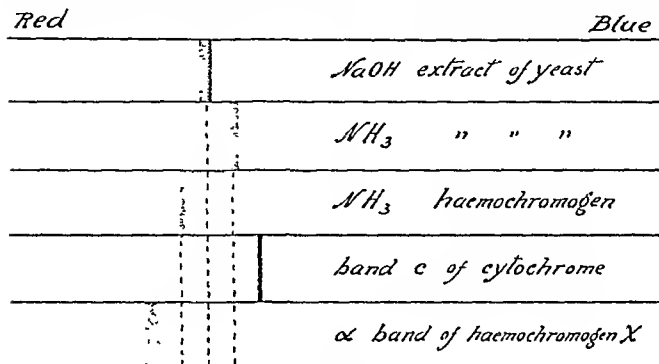


Fig. 3.

are made, the band in question is not always in exactly the same position. And, finally, if the NaOH extract is left for a long time, the band gradually shifts towards the blue. The character of this band, then, and the variability of its position indicate that we are dealing with a variable mixture of two different components.

When yeast is extracted with  $\text{NH}_3$  the situation is more complicated, for  $\text{NH}_3$  is not only an alkali but a nitrogen substance capable of combining with hæm. And, in fact, the spectrum of the  $\text{NH}_3$  extract is different from that of the NaOH extract. The  $\alpha$  band is narrow and sharp and uniform, and it is situated towards the blue of the  $\alpha$  band of the NaOH extract. Thus  $\text{NH}_3$  has not acted simply as an alkali. On the other hand, it has not acted simply as a nitrogen substance, for the  $\alpha$  band of  $\text{NH}_3$ -hæmochromogen itself is to the red of the  $\alpha$  band of the NaOH extract and not to the blue as is the  $\alpha$  band of the  $\text{NH}_3$  extract. The only remaining possibility, then, is that  $\text{NH}_3$  has acted both as an alkali and as a nitrogen substance, and that the  $\alpha$  band of the  $\text{NH}_3$  extract, sharp though it is, is a mixture of the  $\alpha$  band of  $\text{NH}_3$ -hæmochromogen and the  $\alpha$  band of another hæmochromogen, which must be present in any alkaline extract.

The problem is now to reconcile these facts in detail and, in particular, to explain the relative positions of the various bands. First, however, we shall describe an experiment, which throws some light on the nature

of one of the constituents of the NaOH extract. In order to test the hæmochromogen nature of the cytochrome components Keilin bubbled CO through the NaOH extract. The broad uneven band *A* disappeared and was replaced by a faint broad band, more towards the red. On standing, this band split into two bands, one of which was almost too faint to be seen, the other corresponded exactly to band *C* of cytochrome. On still further standing, the spectrum became exactly like the original NaOH spectrum. The explanation is probably this. The uneven band in the NaOH extract is a mixture of the  $\alpha$  bands of two hæmochromogens (*A* and *B*), one of these hæmochromogens (*A*) being identical with the hæmochromogen responsible for the sharp band *C* of cytochrome. The CO bands of these hæmochromogens also fuse. On standing the CO diffuses out of the solution. Hæmochromogen *A* (responsible for band *C*) has less affinity for CO than hæmochromogen *B* and so it loses its CO first and becomes reduced before hæmochromogen *B*. The idea of difference in affinity is due to Keilin. The  $\alpha$  band of reduced hæmochromogen *A* is not influenced by the band of CO hæmochromogen *B*. On further standing hæmochromogen *B* also loses its CO and becomes reduced and the reduced bands of hæmochromogens *A* and *B* now fuse again to give the original spectrum. In agreement with the hypothesis that NaOH leaves the hæmochromogen *A* of cytochrome unchanged, is the fact observed by Keilin that water will extract hæmochromogen *A* from yeast but not hæmochromogen *B*. If now NaOH is added to the water extract the position of the bands remains unchanged.

The relative positions of the bands of the two alkaline extracts can now be explained by three hypotheses (see Fig. 3):

(1) The NaOH extract is a mixture of hæmochromogen *A* of cytochrome and another hæmochromogen *X*. The darker part of the band is due to the band of hæmochromogen *A*.

(2) The  $\text{NH}_3$  extract is essentially a mixture of hæmochromogen *A* of cytochrome and ordinary  $\text{NH}_3$ -hæmochromogen.

(3) The  $\alpha$  band of  $\text{NH}_3$ -hæmochromogen is towards the blue of the  $\alpha$  band of hæmochromogen *X* and is probably sharper than that band. Hence the mixed band of the  $\text{NH}_3$  extract is to the blue of the mixed band of the NaOH extract, and is much narrower and sharper.

These experiments illustrate the point already made that the constituent hæmochromogens of cytochrome must have different properties. The band of hæmochromogen *A* remains unchanged when alkali is added. The  $\alpha$  band of hæmochromogen *B* is shifted either due to an ionisation

caused by the alkali or to a substitution of a different nitrogen compound. And the two hæmochromogens of the NaOH extract apparently have very different affinities for CO.

It looks, from Keilin's observations, as if the transformation from the reserve tissue hæmochromogen to cytochrome caused by drying, is a shift in the hæmochromogen equilibria similar in nature but opposite in direction to that caused by the addition of NaOH to yeast, as if the reserve tissue hæmochromogen, like the NaOH extract, is a mixture of the original hæmochromogen *A* of cytochrome and hæmochromogen *X*. This would mean that in these transformations the nitrogen compound  $N_1$  of hæmochromogen *A* (which is responsible for the sharp band *C* of cytochrome), on the whole remains attached to its hæm molecules, while other hæm molecules are attached sometimes to  $N_2$  and sometimes to  $N_3$ .

We wish to express our indebtedness to Dr D. Keilin, who provided us with much of the material from which we extracted hæm and to Mr H. Munro Fox, who brought actinohæmatin to our attention.

The expenses of this research were paid, in part, out of a grant of the Medical Research Council to Mr Barcroft.

### SUMMARY.

1. Hæmochromogen consists of hæm attached to a nitrogen compound.

2. Many natural pigments which can be converted into hæmochromogen contain different nitrogen compounds but the same hæm.

3. In particular cytochrome, a universal respiratory catalyst, contains hæm.

4. Since hæm is omnipresent one does not have to assume in order to explain the haphazard distribution of hæmoglobin that the ability to synthesise hæm has been developed independently in nature many times.

5. Hæm is in equilibrium with various nitrogen compounds to form some of the hæmochromogen components of cytochrome. Variations in the proportions and nature of these nitrogen compounds are responsible for the variations in the relative intensities and positions of the bands of these hæmochromogens.

6. One two-banded component of cytochrome contains a substance different from hæm though perhaps related to hæm.

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## CORRECTION.

In our paper on hæmochromogen (*This Journal*, 60. p. 62, 1925) we incorrectly stated that Willstätter and his co-workers had shown purified pepsin and trypsin to be identical. What Willstätter, Waldschmidt-Leitz, and Memmer did prove in the article to which we referred (*Ztschr. physiol. Chem.* 125. p. 93, 1923) was that the activity of the pancreatic lipase at various hydrogen ion concentrations is dependent on the "Begleitstoffe" present.

## OBSERVATIONS ON THE PULMONARY CIRCULATION.

### Pulmonary Circulation in the Heart-Lung Preparation.

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MOST of our knowledge of the pulmonary circulation has been derived from experiments performed on the intact animal or on the heart lung preparation. The interpretation of the results obtained on the whole animal are often doubtful on account of the difficulty of determining whether the changes in the pulmonary circulation are primary or secondary to alterations in the systemic circulation. In this respect the heart lung preparation affords better possibilities for the study of the pulmonary circulation because most of the hæmodynamic factors, and especially the systemic resistance and the blood flow, are strictly controlled and can be changed independently of each other. However, just because of the independence of these factors the results obtained with the heart lung preparation cannot be transferred without reservation to the whole animal.

The study of the pulmonary circulation with the use of the heart-lung preparation was begun by Fuhner and Starling<sup>(1)</sup> and continued by Kuno<sup>(2, 3)</sup> and by Straub<sup>(4)</sup>. Most of the other observers made their experiments on the whole animal. [For literature see Wiggers<sup>(5)</sup> and Sharpey-Schafer<sup>(6)</sup>.] The results obtained by these two groups of workers are, in many respects, not concordant, the differences being generally explained by the assumption that the heart in the heart lung preparation is in a hypodynamic state, and thus the whole cardio-pulmonary circulation rendered abnormal.

The experiments described below were performed with the object of re-investigating the mechanical factors which influence the pulmonary circulation. All the experiments were conducted on the heart-lung preparation, but under somewhat more standardised conditions and with consideration of some factors which had previously been neglected.

Dogs were used in all the experiments. The heart-lung preparation was prepared according to the method which has been frequently described by Starling and his collaborators. The animals were anaesthetised with chloralose (0.1 grm. per kilo); defibrinated blood was used in the circulation.

#### A. EFFECT OF CHANGES IN THE SYSTEMIC ARTERIAL RESISTANCE UPON THE PULMONARY BLOOD-PRESSURE.

Most of the observers who worked on the intact animal found no relation between the pressure in the pulmonary artery and that in the systemic circulation. L. Hill(7) and Bradford and Dean(8) consider that only in cases of weakening of the cardiac muscle does a rise in the systemic pressure cause a rise in the pulmonary pressure. It is usual to regard this effect as due to so-called "back-pressure," that is, an increased arterial resistance diminishes the emptying of the left ventricle and causes a retention of blood in the left auricle, a rise of pressure in the latter, and a damming of the blood back into the lungs. Wiggers(5) believes that under normal conditions a back pressure effect is prevented by the improved contractions of the left ventricle, which responds with stronger beats to every increase in its filling and so prevents a rise of pressure in the left auricle. When back pressure does occur this compensatory mechanism is not able to prevent intense pulmonary congestion and a rise in the pulmonary pressure.

The observations made on the heart-lung preparation are in striking contrast with those made on the intact animal. Fühner and Starling found in the heart-lung preparations in which the hearts were in an apparently good condition a close dependence of the pulmonary pressure upon the systemic pressure. In their experiments every rise in the systemic pressure was followed by a corresponding rise in the pulmonary pressure, so that the relation of the pulmonary to aortic pressure varied only slightly between 1 : 6 and 1 : 7.5. Fühner and Starling ascribed their results to back pressure. Straub made similar observations and also explained them at least in part as being due to back pressure.

In our experiments the pulmonary pressure was measured by means of a cannula introduced into the upper branch of the right pulmonary artery close to its entry into the lung. The cannula was connected to a saline manometer, the top of which was in communication with a sensitive piston recorder.

The results of the experiments were conclusive in confirming the observations of Fühner and Starling. Fig. 1 serves as an example



of the effect of an abrupt rise in the systemic blood-pressure upon the pressure in the pulmonary artery. The output of the heart was in this experiment 550 c.c. per minute; an increase in the systemic pressure from 50 to 150 mm. Hg was accompanied by a rise in the pulmonary pressure from 170 to 240 mm. H<sub>2</sub>O, the output of the heart remained constant. The changes in the pulmonary pressure are only approximately proportional to the changes in the systemic pressure. We found much wider variations in the ratio between pulmonary and systemic pressures than Fulmer and Starling. As will be seen later, this ratio depends on several factors, the principal of which is the output of the heart. The output influences in the heart-lung preparation the pulmonary pressure to a much greater extent than the systemic resistance. For any given output of the heart the ratio between the two pressures can vary with increasing systemic resistance as much as from 1:1 to 1:11, bringing this ratio nearer to the figures given by Tigerstedt(9) and Knoll(10) for the whole animal. Successive increases in the systemic pressure are in most cases followed by somewhat smaller rises in the pulmonary pressure, so that the ratio between the two gradually increases.



Fig 1. Effect of a rise in systemic blood pressure upon that in the pulmonary artery. Upper tracing—pulmonary blood pressure. Lower tracing—systemic pressure. Temp 37.5°C

The tracings in this and in the following figures were obtained with a heart lung preparation

Heart lung preparation	Output	580	580	580	580	580	580	580	580
	c.c. per minute,	heart rate	120,	temp	36.8° C				
Mean systemic B. P. (Hg)	50	75	95	105	200	150	120	80	40
Mean pulmonary B. P. (H <sub>2</sub> O)	140	160	182	270	300	250	215	155	120
Ratio=1.	4.85	6.4	7.1	8.1	9.1	8.2	7.6	7.0	4.53

The effect of changes in the systemic pressure upon the pulmonary pressure varies from experiment to experiment and also during the course of each. The dependence of the pulmonary pressure upon the systemic becomes more noticeable as the experiment proceeds. At the beginning of an experiment a change in the systemic pressure from 60

to 100 mm. may cause a rise in the pulmonary pressure of only 20 or 30 mm.  $H_2O$ , while at the end (most experiments continued for 4-6 hours) the same rise in the systemic pressure with the same output of the heart per minute as before, may increase the pulmonary pressure by 150 or even 200 mm. of water. Therefore, the ratio between the two pressures necessarily changes more at the beginning than at the end of the experiment.

From the point of view of the back pressure theory something similar would be expected and explained probably on the lines of progressive weakening of the heart due to experimental procedure. This undoubtedly takes place, but, as will be shown later, it cannot explain the progressive effect of the changes in the arterial pressure upon the pulmonary pressure.

It seems evident that measurements of the changes in the pressure in the pulmonary artery, and in the aorta, though they show a certain parallelism, do not demonstrate the mechanism by which this parallelism is brought about and do not decide the question in favour of or against "back pressure." Only simultaneous measurements of the changes in pressure in the left auricle and in the pulmonary artery can decide whether back pressure is the determining factor. The pressure in the left auricle was measured in our experiments in the same manner as the pulmonary pressure: the cannula was introduced through the left auricular appendix. As the result of very numerous observations we feel convinced that in a normal heart-lung preparation, when the systemic pressure is increased the auricular pressure in any given case behaves in a manner so different from the pulmonary pressure that the back pressure theory must be either entirely abandoned or regarded as of very minor importance.

During the greater part of an experiment, while the heart and the whole preparation is in a good condition, elevations of the systemic pressure cause no or hardly any change in the mean auricular pressure: the pulmonary pressure, nevertheless, invariably shows a rise. Fig. 2 serves as an illustration of this: the auricular pressure at first increased slightly but soon returned to its original level, whilst the pulmonary pressure remained high.

It seems that in such instances, and they are the most frequently observed, no explanation on the grounds of the back pressure theory is possible. The increased strength of each ventricular contraction is sufficient to expel the blood against the higher aortic pressure, and so the accumulation of the blood in the auricle is prevented. In some cases it

was observed that the auricular pressure was even slightly decreased though the pressure in the pulmonary artery remained higher than

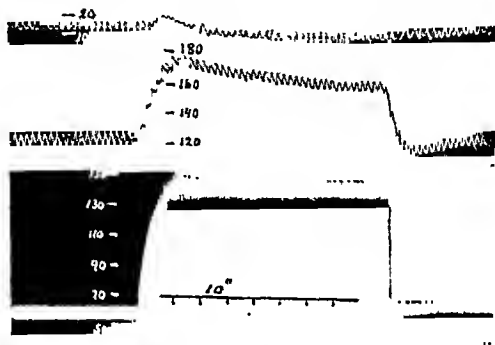


Fig. 2. Effect of rise of systemic pressure on the pressure in the left auricle (upper tracing), and on that in the pulmonary artery (middle tracing). Temp. 37° C. Systemic output 612 c.c. per min.

before. Most careful adjustments of the cannulae and variations in the rate of the rise of the systemic resistance made it highly improbable that these falls in the auricular pressure could be due to mechanical errors. This paradoxical effect of the increase in the systemic pressure upon the auricular pressure, which is occasionally observed, cannot be explained by a weakening of the right ventricle since the pulmonary arterial pressure goes up and remains high throughout the time the aortic pressure is maintained at a high level. Moreover, this phenomenon is observed only with small or moderate outputs of the heart and only at the beginning of an experiment when the condition of the heart is good.

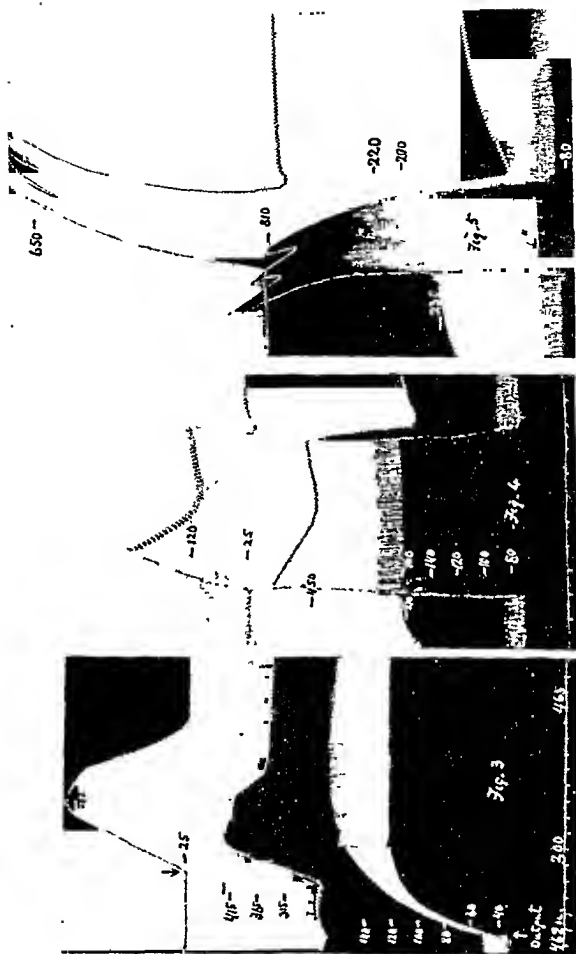
The explanation which we think the most probable lies again in the increased strength of the contraction of the heart. The heart starts each beat with a longer length of fibres and on account of the higher aortic pressure it has a better coronary supply: thus the strength of contraction and the nutrition of the heart are improved, and the auricle has a better chance to expel some of its blood into the ventricle. The following experiment gives an example of this paradoxical fall in the auricular blood-pressure.

Heart-lung preparation. Systemic output: 480 c.c. per min. Temp. 36.5° C. Heart rate: 114. Weight of heart: 57 gms. The systemic blood-pressure was maintained at each level for not less than 5 minutes.

Systemic B.-P. (Hg)	Pulmonary B.-P. (H <sub>2</sub> O)	B.-P. in left auricle (H <sub>2</sub> O)
50	135	47
120	200	38
40	140	45
100	185	37
150	220	30
200	280	39
150	210	30
40	124	50

The heart in this experiment was in an excellent condition; the small fall in the auricular pressure is evident until the systemic pressure reaches the high level of 200 mm. Hg, when the auricular pressure increased slightly.

These cases are, however, comparatively rare. In most cases rises in the systemic pressure with a moderate cardiac output per minute cause hardly any change in the auricular pressure. This holds good for a wide range of increments of the aortic pressure. Beyond a certain limit, which varies with the condition of the heart, the auricular pressure becomes more and more affected by the elevation of the systemic pressure. With progressively increasing arterial resistance the mean pulmonary and auricular pressures exhibit in the great majority of cases the following changes. At first the two pressures behave as already described, namely, the pulmonary pressure always increases while the auricular does not change or even falls slightly. In most experiments the auricular pressure remains unchanged until the aortic resistance reaches about 150 mm. Hg. At the same time the pulmonary pressure increases more or less steeply to a new level, at which it remains as long as the systemic pressure is kept constant. With further increases in the systemic pressure to about 190 to 200 mm. Hg the pulmonary and auricular pressures behave as in Fig. 3. The auricular pressure after a temporary increase returns to its original level; this temporary change in the auricular pressure is very nearly duplicated by the pulmonary pressure, but this latter after the temporary "overshot" has receded, assumes a level which is higher than the original. With still further rises of the systemic pressure the auricular pressure becomes more and more affected; it also shows a smaller tendency to recovery and now assumes a level which is somewhat higher than the original level (Fig. 4). The higher the rise of the systemic pressure the smaller the recovery of the left auricular pressure, and finally when the systemic resistance reaches a very high



Figs. 3, 4, 5. Varying degree of back pressure produced by rise in systemic pressure (see text). Upper tracing—auricular blood pressure. Middle tracing—pulmonary blood pressure. Lower tracing—systemic pressure.

level the pressure in the auricle suddenly begins to rise without any tendency to recover (Fig. 5). The systemic output of the heart, which is unchanged throughout the experiment, now becomes diminished and the heart shows extreme dilatation. The pulmonary pressure also, with each successive rise of the aortic resistance, shows more and more pronounced "overshots," and then suddenly sometimes a small further rise in the systemic pressure causes, like the bursting of a dam, a large rise in the pulmonary and auricular pressures without any tendency to recovery. The better the condition of the heart the higher must be the rise in the systemic pressure to cause the effects presented in Figs. 3, 4 and 5. In hearts which are weakened by experimental manipulations even small rises of blood-pressure cause an effect similar to that shown in Fig. 4, while that shown in Fig. 5 will be caused at the end of an experiment by a comparatively smaller rise of the resistance than at the beginning of the experiment. In a number of experiments the big final rise could not have been produced in the first half of an experiment even with increases in the aortic pressure as high as 300 mm. Hg. In some experiments these three different types of effects were more evident than in others.

The important feature of these observations is that except in the case of an excessive rise of the arterial resistance the auricular pressure is always affected to a smaller extent than the pulmonary pressure, so that even when the auricular pressure does rise this cannot wholly account for the rise in the pulmonary pressure.

The temporary "overshots" of the pressure, when they do occur, affect both the left auricular and pulmonary pressure to exactly the same extent. The overshoots in both cases can well be explained by back pressure effect: they must be considered as signs of a temporary failure of the heart to expel the blood. Owing to the increased force of contraction of the left ventricle and probably also the left auricle, the heart frees itself from the excess of blood and both the left auricular and pulmonary pressures fall, the first to approach the original level, the second to a level above the original. If the volume of the ventricles is measured simultaneously with the pulmonary and the auricular pressure, it can be seen that an abrupt rise in the systemic resistance often causes a similar overshoot in the plethysmographic curve. This fact has been known and described before.

The extent to which the pulmonary pressure is affected by a given rise in the arterial resistance is also dependent upon the output of the heart. The bigger the output the more is the effect accentuated.

In hearts which are not in good condition, and which show an early rise in the auricular pressure, the effect of increased output is somewhat different. At first with small outputs it is only the pulmonary pressure which is affected, the auricular remaining unchanged. With a larger flow the same increase in the arterial resistance raises also the auricular pressure but always to a smaller degree than the pulmonary pressure. The difference in the absolute rise in the two pressures becomes smaller with increased output, and finally with very large outputs the same rise in the arterial resistance will affect the auricular pressure to a greater extent than the pressure in the pulmonary artery, exhibiting all the signs of regurgitation of blood. At the same time, the systemic output falls and the heart becomes insufficient. It is difficult to reach such a state in a heart which is at all normal.

The experiments just described show that in the heart-lung preparation it is very difficult to produce back pressure. Even when temporarily produced, as evidenced by the overshoot of the pressure in the auricle, the heart can in a few beats accommodate itself to the new conditions, and by increasing the strength of its contractions prevent back pressure as well as regurgitation of the blood into the pulmonary system.

#### B. THE PART PLAYED BY THE CORONARY BLOOD FLOW IN THE PULMONARY CIRCULATION.

Since there is no evidence of an active constriction of the pulmonary blood vessels when the systemic resistance is increased, the observed rise in the pulmonary pressure must necessarily mean that their filling is increased with each rise in the aortic pressure and diminished when the pressure in the aorta falls. Strauh<sup>(1)</sup>, who has demonstrated that in the heart-lung preparation there is a considerable pulmonary hyperæmia whenever the aortic pressure is increased, regards it as being due primarily to back pressure, which we have seen is not the case, but he also suggests another factor. This second factor Strauh finds in the increase of the coronary blood flow whenever the aortic pressure is raised. Since in the heart-lung preparation the inflow of the blood into the heart is constant and, within wide limits, independent of the arterial resistance, the additional amount of blood from the coronaries must increase the filling of the right heart and augment the output of blood into the pulmonary artery. Straub did not verify this explanation by experiment and regards the increase in the coronary blood flow as being of minor importance.

level the pressure in the auricle suddenly begins to rise without any tendency to recover (Fig. 5). The systemic output of the heart, which is unchanged throughout the experiment, now becomes diminished and the heart shows extreme dilatation. The pulmonary pressure also, with each successive rise of the aortic resistance, shows more and more pronounced "overshots," and then suddenly sometimes a small further rise in the systemic pressure causes, like the bursting of a dam, a large rise in the pulmonary and auricular pressures without any tendency to recovery. The better the condition of the heart the higher must be the rise in the systemic pressure to cause the effects presented in Figs. 3, 4 and 5. In hearts which are weakened by experimental manipulations even small rises of blood-pressure cause an effect similar to that shown in Fig. 4, while that shown in Fig. 5 will be caused at the end of an experiment by a comparatively smaller rise of the resistance than at the beginning of the experiment. In a number of experiments the big final rise could not have been produced in the first half of an experiment even with increases in the aortic pressure as high as 300 mm. Hg. In some experiments these three different types of effects were more evident than in others.

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The extent to which the pulmonary pressure is affected by a given rise in the arterial resistance is also dependent upon the output of the heart. The bigger the output the more is the effect accentuated.



cannula is followed by a rapid fall in the pulmonary pressure, and the higher the arterial pressure the bigger is the fall of pulmonary pressure. The introduction of the coronary cannula cannot be performed, however, without a certain mechanical manipulation of the heart, and this may possibly have some effect on the pulmonary pressure.

The removal of the cannula is much easier and does not require more than a second at the utmost. When the cannula is removed the pulmonary pressure rapidly returns to its original level. Fig. 6 illustrates the effect

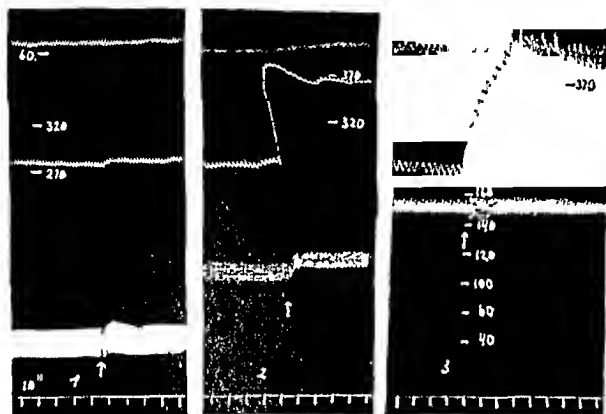


Fig. 6. Effect of removal of the coronary cannula upon the blood pressure in the pulmonary artery. From top downwards pressures in the left auricle, pulmonary artery and the systemic blood pressure. Total output throughout about 640 c.c. per min. Coronary cannula removed at arrows. Note that the pulmonary blood pressure does not change in spite of the increase in the arterial pressure. The removal of the coronary cannula has a greater effect upon the pulmonary blood pressure at high systemic pressures than at lower ones.

1	Arterial blood pressure	10 mm	Coronary blood flow	36 c.c.
2	"	110 "	"	142 "
3	"	150 "	"	156 "

of the removal of the coronary cannula with increasing arterial resistance: the curves were taken from the second half of the same experiment. The removal of the coronary cannula is nothing more than a sudden increase in the inflow of blood into the right heart and pulmonary artery. Therefore the same changes in the pulmonary pressure should be observed

if the inflow from the reservoir is changed by an amount equal to the coronary blood flow. Fig. 7 shows that this is actually the case. In this

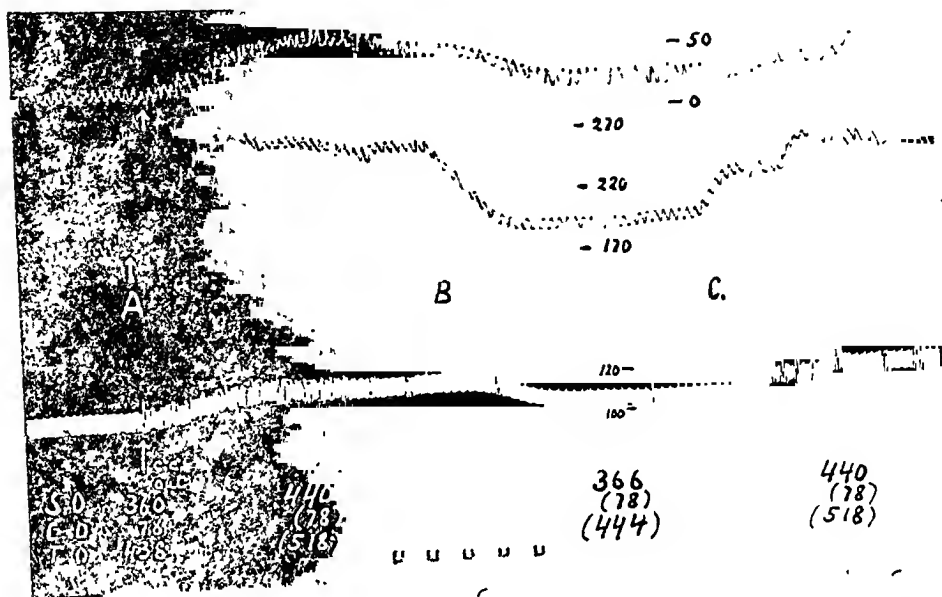


Fig. 7. From top downwards: left auricular, pulmonary and systemic blood-pressures S.O., systemic output; C.O., coronary output; T.O., total output. Figures in brackets are calculated on the basis of the previous determination of the coronary blood flow. Other explanations in text.

case at A the coronary cannula was removed, which meant an increase in the inflow into the right heart by 78 c.c. of blood per minute, raising the total inflow from 438 c.c. to 518 c.c. This increase in the inflow raised the pulmonary pressure from about 190 to 250 mm. of water. At B the inflow from the reservoir was diminished by an amount approximately equal to the coronary blood flow, bringing the total output of the heart again to 444 c.c., and the pulmonary pressure returned to 190 mm. of water. An increase of the total output of the heart to 518 c.c. at C caused the pulmonary pressure to rise again to about 250 mm.

*Effect of changes in the arterial resistance with drainage of the coronary blood upon the pulmonary pressure.*

It should be remembered that a cannula introduced into the coronary sinus drains away about three-fifths of the total coronary blood (13), so that the increment in the total cardiac output produced by increased arterial resistance cannot be entirely abolished by means of the sinus

caunula. Nevertheless, even with the withdrawal of three-fifths of the coronary blood, the effect of increased arterial resistance upon the pulmonary pressure was different from that observed normally with the coronary circulation intact.

The following experiment gives an example of the behaviour of the pulmonary pressure under different arterial resistances, with a constant inflow from the venous reservoir, first with the coronary circulation intact, and then after the introduction of the coronary cannula.

Heart-lung preparation. Systemic output: 588 c.c. per min. Heart rate: 126; temp. 36.8° C.

Before introduction of the coronary cannula.

Systemic B.P. (Hg.)	40	90	150	230	160	100	75	40
Pulmonary B.P. (H <sub>2</sub> O)	165	195	250	340	245	190	175	160

After introduction of the coronary cannula.

Systemic B.P.	40	80	140	220	175	90	70	40
Pulmonary B.P.	150	150	175	190	180	150	150	150
Systemic output	560	534	480	336	440	506	524	560
Coronary flow	26	48	116	198	156	72	52	20

As will be seen from the experiment described above the effect of increased resistance on the pulmonary pressure is very greatly reduced after the introduction of the coronary cannula and draining of the three-fifths of the total coronary blood flow. The degree to which the coronaries participate in the rise in the pulmonary blood-pressure can also be demonstrated in another way. In the experiment from which Fig. 8



Fig. 8. P. ...  
blood. ...  
Figures in brace  
coronary blood

lowwards: left auricular,  
out; C.O., coronary out  
the basis of the pro  
cannula is removed

was taken the coronary flow was measured (*A*) first at an arterial resistance of 55 mm. and then at a resistance of 130 mm. The blood-pressure was then reduced and the coronary cannula removed. The blood-pressure was now raised again to the same high level as before (*B*). This meant an addition of 75 c.c. of coronary blood per min., and, since the inflow from the reservoir was constant, an increase in the total output from 445 c.c. to 520 c.c. per min. A reduction of the output by approximately the amount equal to the increase in the coronary flow (*C*) brought the pulmonary blood-pressure exactly to the same level as it was before the increase in the arterial resistance. At *D* the output was again increased and at *E* when the blood-pressure was reduced to 55 mm. the pulmonary pressure returned to the level existing before the increase in the output at *D*. Experiments of this sort show once more that the increase in the coronary blood flow is sufficient to account for the changes in the pulmonary blood-pressure.

However, drainage of the blood flowing from the coronary sinus does not always entirely abolish the increase in the pulmonary blood-pressure. This rise in pressure is accounted for by the remaining two-fifths of the coronary blood, which flows into the right auricle through the vena Thebesii and the posterior cardiac vein. In de Barenne's (14) modification of the heart-lung preparation the entire coronary blood can be collected, and at the same time the inflow into the pulmonary artery can be maintained constant. By using this preparation we found that even with increases of aortic resistance to the very high levels at which all the systemic output is switched back into the coronary arteries, the pulmonary pressure does not rise at all. The auricular pressure in almost every experiment fell slightly, which brings these experiments into the same group as those described in the beginning of the paper. The fall in the auricular pressure was observed in the experiments without the use of the coronary cannula only in exceptional cases: it was much more frequent with the use of the coronary cannula and was of constant occurrence with de Barenne's preparation. We believe that the strengthening of the contraction of the left ventricle and the more efficient emptying of the left auricle are the main cause of the fall. The following is an example of an experiment with de Barenne's preparation:

It should be noted that the lung preparation. Output between 560 and 572 c.c. per min.					
sinus drainage		Before de Barenne's modification.			
so that the a.p.		60	120	60	150
arterial resistance		190	240	200	270
B.-P.		25	28	25	20
B.-P.					28

After the establishment of de Barenne's modification

Systemic B P	60	100	130	150	60	40	180	40
Pulmonary B P	200	200	196	196	200	200	190	200
Left auricular B P	28	20	12	0	25	30	0	28

The total output of the heart after the establishment of the de Barenne's modification was kept between 560 and 590 c c per min

Patterson and Starling and Kuno have shown that the mean right and left auricular pressures vary only slightly with comparatively large changes in the filling. This must be the reason why the increase in the total output of the heart can manifest itself in an increased pulmonary pressure without affecting the auricular pressure. When the systemic resistance is increased to such an extent that the auricular pressure is also raised, it is due to the increase in the total output of the heart, and to the increased inflow into the auricle and not to diminished emptying of the auricle into the ventricle. This explanation of the changes in the auricular pressure rests on two observations: (1) the changes in the auricular pressure brought about by increased aortic resistance can be imitated by changes in the inflow of the heart which are equal to the variation in the coronary blood flow, (2) after introduction of the coronary cannula the permanent changes in the auricular pressure are absent or converted into a fall. However, the draining of the coronary blood does not abolish entirely the described "overshots," though it greatly reduces them, so that these temporary rises may to some extent be explained by back pressure. The big final rise in the pulmonary and auricular pressures may be observed also after the introduction of the coronary cannula, this shows that the effect is produced mainly by back pressure and regurgitation of the blood into the pulmonary system.

The augmentation of the effect of increased aortic resistance upon pulmonary pressure as the experiment proceeds is not due to progressive weakening of the heart, since at any stage of the experiment the draining of the coronary blood prevents the pulmonary pressure from reacting to changes in the aortic pressure. Even at the end of an experiment, when a rise of the arterial resistance by 50 mm Hg caused a rise in the pulmonary pressure of 190 mm H<sub>2</sub>O, after the introduction of the coronary cannula and draining of three-fifths of the total coronary blood, the same rise in the aortic pressure caused a rise in the pulmonary pressure of only 35 mm H<sub>2</sub>O. In the first place the left auricular pressure increased by 25 mm of water; in the second it fell 15 mm. Hilton and Eichholtz(15) demonstrated that for some yet unknown reason the coronary blood vessels undergo a dilatation with the progress of the

experiment, the coronary blood flow increases and becomes more susceptible to changes in the arterial resistance. This also explains the increased dependence of the pulmonary pressure upon the arterial resistance. It is not that back pressure is now more easily produced, but that the total output of the heart and hence the pulmonary blood flow is increased to a greater extent with every rise in the arterial resistance at the end of an experiment than at the beginning. The weakening of the heart muscle is manifested only by the greater ease with which the final large rise and dilatation of the heart can be produced.

*Direct measurement of the total output of the heart.*

Introduction and removal of the coronary cannula and the use of de Barenne's preparation, in which the right side of the heart does no work, may be thought to render the heart abnormal and so vitiate the results. We therefore decided to measure the total pulmonary flow by a direct method. The apparatus used for this purpose is shown in Fig. 9. It is a simple strohmuhr of the type used by Pavlov and Stolnicov for the measurements of the systemic output.

It is evident that the difference in the blood flow through the pulmonary artery and through the aorta must be at any given time equal to the coronary blood flow. Inequality of contractions of the two ventricles cannot be maintained for a long time since the inflow from the reservoir is maintained constant. Any such unequal contraction would lead to accumulation of blood in one or other of the ventricles and soon lead to dilatation of the heart. In our experiments the flow through the pulmonary artery was always larger

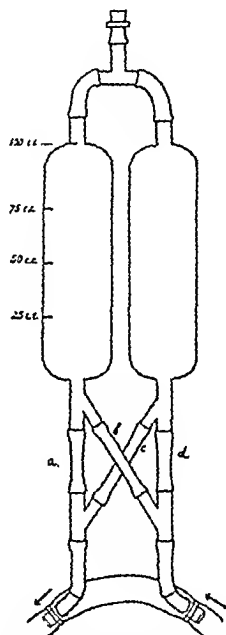


Fig. 9. Diagram of the strohmuhr used for the direct determination of the total output of the heart. One bulb is filled with liquid paraffin, the other with defibrinated blood. The two cannulae are tied in the pulmonary artery. Tubes *c* and *d* are clamped and the blood flows along *b* and *a*. To measure the blood flow tubes *b* and *c* or *a* and *d* are clamped in pairs, the blood enters either one or the other bulb, displaces the paraffin and drives an equal amount of blood from the second bulb into the peripheral end of the pulmonary artery. The deviation of the blood flow from its usual course *b-a* to the bulbs has no effect upon the pulmonary blood-pressure.

in our experiments the flow through the pulmonary artery was always larger

than through the aorta. Only during and a few beats after a sudden fall in the arterial pressure did the aortic flow exceed the pulmonary flow. During this time the coronary flow becomes reduced, while the lungs still contain a large amount of blood, maintaining the inflow into the left heart above that into the right heart. We obviated any error due to this factor by taking the measurements of the blood flows not before one full minute after any rise or fall in the aortic resistance. The results of one of the experiments is given in the diagram (Fig. 10).

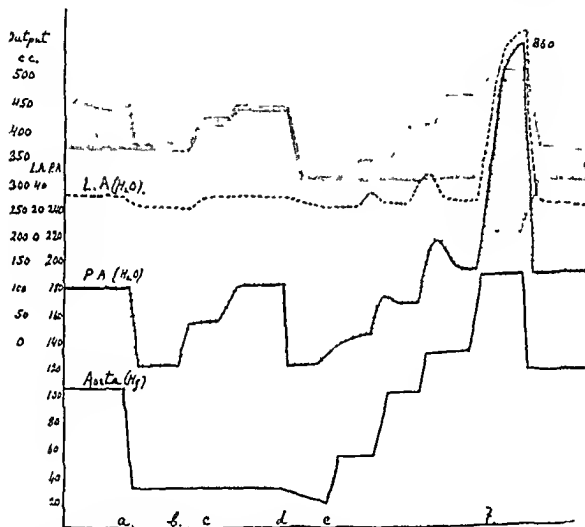


Fig. 10. The lower margin of the shaded area represents the systemic output of the heart; the upper margin—the total output as determined by the stromuhr inserted into the pulmonary artery. The width of the shaded area represents the total coronary blood flow. The reduction of the systemic pressure at *a* does not affect the systemic output but reduces the total output and the pulmonary pressure. At *b* and *c* the systemic pressure is kept constant but the inflow from the venous reservoir is gradually increased until the total output reaches the level at which it was during the high systemic pressure. The pulmonary pressure returns to its previous level, showing that it is dependent upon the arterial resistance only in so far as the latter influences the total output. At *d* the inflow is diminished and between *e* and *f* the arterial pressure is once more increased. Temporary back pressure effects are shown by the "overshots" of the pulmonary and the left auricular pressures. A final rise of the arterial pressure is followed by a pure back pressure effect with a considerable fall in the systemic output of the heart.

The results of all these experiments demonstrate that the main factor which determines the pulmonary pressure is the blood flow, and that the arterial resistance acts only so far as it affects the output of the right ventricle. Back pressure does not play any part in the rise in the pulmonary blood-pressure, at any rate not until the mitral valves are rendered insufficient so that regurgitation of blood into the auricle takes place.

#### SUMMARY.

1. The total output of the heart as distinguished from the systemic output, in the heart-lung preparation with any constant venous inflow varies directly with changes in the arterial resistance, and is greater the higher the resistance.

2. These changes in the total output are entirely accounted for by the changes in the coronary blood flow.

3. The pulmonary arterial blood-pressure in the heart-lung preparation with a constant venous inflow varies directly with changes in the aortic resistance.

4. The changes in the pulmonary pressure are not due to "back pressure" effects but to the increased total output of the heart, *i.e.* increased coronary blood flow.

5. Back pressure takes place only when the heart becomes very much dilated and blood regurgitates from the ventricle into the left auricle.

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## THE RELATION OF OVARIAN FUNCTION TO MENSTRUATION. BY WILFRED SHAW

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London )

ALTHOUGH very considerable advances have been made in elucidating the significance of menstruation, the correlation of the cyclical changes in the ovaries with the periodic changes in the endometrium has not received the attention it deserves. It is generally accepted that menstruation is controlled by the ovaries, for if the latter are removed in the child-bearing period menstruation ceases, and there is a great deal of evidence to suggest that the corpus luteum is in some way related to this process. The aim of this research was to test the work of R Meyer and of Schroder in order that, if confirmed, the principles they deduced might be applied to the investigation of pathological conditions of the ovaries when diseases of the uterus are present.

The method employed was histological and its object was to examine the endometrium at various phases of the menstrual cycle, particularly during menstruation, to examine the ovaries for corpora lutea, to compare these histological results with the menstrual history and in this way to obtain any relation which may exist between the ovaries and the endometrium.

In this paper, the histology of the endometrium will first be described then the changes in the ovaries in the menstrual cycle will be reviewed, and lastly, the relation between the two will be discussed.

### *The Endometrium*

In 1908, Hitschmann and Adler<sup>(1)</sup> carried out their classical research on the human endometrium. They compared the histological structure of the endometrium with the phase of the menstrual cycle and showed that its form varied and that this variation was related to the phase of the cycle. They suggested that four phases of the menstrual cycle should be recognised, viz the post-menstrual, the interval, the pre-menstrual and the menstrual, and pointed out that although no hard-and-fast line differentiated one phase from the next, the characters of the endometrium during each phase were well defined. Schroder<sup>(2)</sup> has given a very detailed description of the histology of the endometrium and Sekiba<sup>(3)</sup>, Novak and Te Linde<sup>(4)</sup> have described the changes

in various phases. Heape<sup>(5)</sup> has described the histology of the endometrium during menstruation in monkeys. In my own investigations the main objects were to determine the time when the pre-menstrual changes are first observed, and to investigate the endometrium during menstruation: but as the cyclical changes are not widely known I will refer to them as well.

My material was derived from curettings and from the endometrium of the uterus removed by operation. Over 100 specimens have been examined, but the majority of these were either grossly abnormal or were obtained from patients who were menstruating irregularly and consequently were useless for my purpose. I have had some 40 specimens which showed no pathological lesions from patients who were menstruating regularly and this is the material I have used. I have found Schröder's technique of immediate fixation in 95 p.c. alcohol to be the best method of fixation, but I pass the specimens through carbon bisulphide instead of xylol. As a routine I prepare three slides, staining the sections with Pappenheim, hæmatoxylin and eosin, and hæmatoxylin and mucicarmine. In each case the menstrual rhythm was known, together with the date of the last menstrual period. The normal cycle is one of 28 days and the first day of the period of bleeding is taken as the first day of the cycle. The phase of the cycle is reckoned in days, and not in the manner suggested by Hitschmann and Adler, so that as the day of the operation was known, the specimen was referred to as corresponding to a particular day of the cycle.

The endometrium of the body of the uterus consists of a surface epithelium, glands and a stroma. On the 10th day of the cycle, that is to say, ten days after the beginning of the last menstrual period, the endometrium has the following structure. The cells of the surface epithelium are low columnar: the glands are simple tubules which take a slightly sinuous course through the stroma at right angles to the surface epithelium. The cells lining the glands are low columnar with their nuclei lying at the base, and the inner border of the cells forms a regular unbroken line. The stroma consists of blood vessels, lymphatics and stroma cells. The vessels are thin-walled and are not conspicuous at this stage. The stroma cells in the main take the form of spindles, but round cells are also present; they contain but little protoplasm and on the 10th day are not swollen. The form of the endometrium on the 10th day is to be taken as the resting condition of the uterine mucosa. It resembles the structure of the latter after the menopause and at this phase no signs of activity can be found.

The earliest signs of activity are observed about the 14th day in the form of mitoses in the nuclei of the cells lining the glands. Schroder and Novak state that these mitoses can be seen earlier than the 14th day, but apart from the mitoses seen in the regenerating epithelium in the post-menstrual phase, I have not observed mitoses prior to the 14th day. Between the 14th and 17th days the appearance of the endometrium is as follows. The cells of the surface epithelium are more columnar and their protoplasm contains mucicarmine positive drops. The glands hypertrophy, become more sinuous and their acini become dilated. The cells lining the glands become more columnar and mucicarmine positive drops appear in their protoplasm. Characteristically at this phase is the appearance of bright translucent areas behind the nuclei which push the latter into the middle of the cells. The inner border of the cells becomes a little irregular and a thin ring of mucicarmine positive material lies immediately adjacent to it within the cavity of the gland. The stroma is oedematous and its cells are swollen.

Between the 17th and 20th days hypertrophy of the whole endometrium continues. The glands become more sinuous and more dilated and their alveoli contain large amounts of mucicarmine positive material. The cells of the glands retain their mucicarmine positive drops and at this stage the bright areas can be seen advancing towards the cavity of the gland to be discharged into the debris there. The result is that the cells lose their columnar form and appear flattened out by the secretion within the gland. At the end of this stage the nuclei have returned to their basal position but the inner border of the cells is completely irregular. The stroma is oedematous with the oedema localised to the middle zone of the endometrium. The stroma cells are swollen and the capillaries are dilated.

After the 20th day, in addition to the general hypertrophy, other characters are displayed. The outer borders of the glands become crenated and small lateral invaginations into the lumina are seen. The cells have lost their translucent areas and tend to be flattened out by the pressure within the gland cavity. Their inner border is irregular and merges into the mucous secretion in the lumen of the gland. The stroma is now differentiated into three layers. Immediately beneath the surface epithelium the swollen stroma cells are packed closely together to form a dense layer surrounding the ducts of the glands. Although this layer is best seen just before menstruation it can be identified on the 20th day. The middle zone which surrounds the dilated glands is very oedematous, the stroma cells are here widely separated

but the capillaries are dilated. In the basal zone no hypertrophy of the glands occurs and the stroma is not cedematous. As Schröder pointed out, the basal zone of the pre-menstrual endometrium differs but little from the endometrium of the post-menstrual phase.

Between the 20th and 28th days the hypertrophy continues with the specific pre-menstrual changes becoming better marked. Immediately before menstruation the endometrium resembles very closely the young decidua of early pregnancy. The superficial zone with its closely packed swollen stroma cells represents the compact layer of the decidua. The middle zone with its dilated glands and cedematous stroma resembles the spongy layer. If pregnancy supervenes, whether as a uterine or an ectopic gestation, the decidua is formed and the decidua represents a further stage in the development of the pre-menstrual changes. Because a uterine decidua is found in cases of ectopic gestation it must be assumed that the factor controlling decidual reaction is not the local stimulation of the imbedded ovum. Again, it is well known that decidua-like tissue is found in the pelvis at a distance from the uterus in cases of pregnancy. Recently Schiller (6) has shown that decidual cells can be found in the ovaries and in the peritoneum in cases where no pregnancy exists. His paper should be consulted for the literature on the distribution of ectopic decidua in cases of uterine pregnancy. It follows from these considerations that the production of decidual tissue because of its wide and irregular distribution must be controlled through the blood stream.

If pregnancy does not supervene, menstruation occurs and the changes in the endometrium during menstruation have now to be dealt with. I have been fortunate in obtaining three specimens of the endometrium on the first day of menstruation. Well-defined changes which are easily recognised can be seen. The most prominent feature is the disorganisation and disintegration of the glands of the superficial and middle zones of the endometrium. The epithelial cells become separated from each other to be scattered amongst the stroma and to fill the lumina of the glands. In addition they stain badly. The stroma is infiltrated with lymphocytes and polymorphonuclear cells particularly in the superficial zone. Plasma cells are few and interstitial hæmorrhages are scanty, though usually a small layer of hæmorrhage is seen immediately beneath the surface epithelium and in small areas the latter is shed. In the basal zone the endometrium retains its original form. These appearances on the first day of the period of bleeding are those of a degenerative process. It seems that the hæmorrhage is produced as a secondary factor to this degeneration—produced perhaps by a

degeneration of the endothelium of the capillaries. It has been shown by O. Frankl and Aschner (7) that the menstrual fluid contains a tryptic ferment. It is possible that this ferment is responsible for the degeneration of the endometrium and of the endothelium of its capillaries.

These degenerative processes result in the shedding of portions of the superficial layers of the endometrium. This is borne out by the examination of clots found in the cavity of the uterus which is removed during menstruation. In one of my cases hysterectomy was performed on the second day of menstruation. The uterus was immediately incised and several clots were found lying free in the cavity and not attached to the endometrium at any spot. These clots were then examined histologically and were found to contain degenerate glands similar in form to the pre-menstrual glands of the endometrium, swollen cells resembling the swollen stroma cells of the pre-menstrual endometrium and blood cells. In another case where death had taken place on the 3rd day of menstruation, the uterus was carefully excised post mortem so that no opportunity occurred for a piece of endometrium to be detached in the process of removal, and was found to contain a clot which again consisted of degenerate glands, stroma cells and blood clot. In addition to these cases I have had several specimens of smetings taken from patients who were menstruating at the time of operation, which contained degenerate glands, the histological appearance of which indicated that they were lying free in the cavity of the uterus. These observations on the shedding of the endometrium during menstruation agree with the work of van der Leyen (8), Lindner (9), Sekiba (3) and Novak and Te Linde (4).

The next question to decide is the amount of endometrium which is shed. It is probable that the greater part of the superficial and middle zones of the pre-menstrual endometrium is shed, for if sections are made through the whole wall of the uterus towards the end of the period of bleeding, the endometrium is represented by the basal layer alone and no surface epithelium remains. The glands open widely into the cavity of the uterus and the stroma is bare. This method of investigation must be pursued with care, for unless the sections are made exactly at right angles to the surface of the endometrium, they will not necessarily include the superficial portion of the latter. The endometrium at the end of the period of bleeding consists only of the basal layer found in the pre-menstrual phase. After menstruation, repair and regeneration of the endometrium occur, the epithelium of the glands growing over and covering the bare stroma. This process of recovery is seen clearly in

curettings obtained immediately after menstruation. It has already been emphasised that the basal layer of the pre-menstrual endometrium resembles very closely the condition of the endometrium in the post-menstrual phase. Consequently, after the production of a new surface epithelium the endometrium attains its resting form and this persists with but little hypertrophy until the pre-menstrual changes commence again about the 14th day.

These cyclical changes agree very closely with Heape's account(5) of the histology of the process of menstruation in monkeys. In the human endometrium the cyclical changes can be readily identified with specimens appropriately stained. It is possible to state to within a few days the phase of the menstrual cycle at which the operation was performed. Further, I have never found pre-menstrual changes in specimens obtained in the post-menstrual phase and specimens obtained in the second half of the menstrual cycle invariably show the specific pre-menstrual changes, provided the woman is menstruating regularly. It follows from these considerations that activity in the endometrium occurs about the middle of the cycle, and that if pregnancy supervenes the pre-menstrual changes become better marked and result in the production of the decidua. If, on the other hand, menstruation occurs, the pre-menstrual endometrium degenerates, the greater part of it is shed and the dilated capillaries are opened up to produce the menstrual hæmorrhage. In the post-menstrual phase repair and regeneration take place and until about the 14th day no signs of activity are displayed.

*The cyclical changes in the ovaries.*

If the ovaries are removed from a woman in the child-bearing period but the uterus retained, menstruation usually ceases. Exceptions occur, but Bland Sutton suggested that they might be explained by assuming either incomplete removal of the ovaries or the existence of an accessory ovary. It follows that the cause of menstruation must lie in the ovaries.

It is known from investigations on lower animals that a Graafian follicle ripens, then ruptures to discharge its ovum and afterwards becomes converted into a corpus luteum. In the human ovary it is easy to demonstrate ripening follicles, and specimens of early human corpora lutea have been described, particularly by Robert Meyer(10) and Novak(11), so it is reasonable to assume that the same processes take place in Man. As the age of a corpus luteum can be deduced fairly accurately from its histological structure (cp. p. 201), and as in my series of 22 cases obtained from women who were menstruating regularly, in

each case a corpus luteum was found less than 28 days old, it follows that ovulation occurs once a month. In human beings the time when ovulation takes place is disputed and a great deal of confusion exists as to the structure and function of the corpus luteum. It was in an attempt to elucidate these problems that my own researches were undertaken. The method I employed was histological and served to deal with both problems.

It is clear that if a young corpus luteum is found the phase of the cycle when the specimen was obtained corresponds fairly closely with the time of occurrence of ovulation. With human material there seems no other reliable method of deducing the time of incidence of ovulation. Consequently this point will be cleared up by finding specimens of early corpora lutea. It is frequently stated that ripening follicles and young corpora lutea can be identified with the naked eye. I am convinced that this is impossible. It is perfectly easy to mistake atretic follicles and follicular cysts for young corpora lutea and blood cysts and old corpora lutea may be mistaken for the most recent corpus luteum. The only reliable method with human material is the complete histological examination of both ovaries. In this way the youngest corpus luteum will be identified microscopically, and with a series of such specimens of known dates the development and structure of the corpus luteum can be traced. In Corner's (12) words "The only hope of trustworthy results in this problem depends upon the possession of an unbroken series of normal specimens of known ages."

My material was derived from 22 cases. With one exception an accurate menstrual history was obtained in each case. The specimens were removed at operation and immediately fixed in neutralised formalin. In each case the patient was menstruating regularly and as, with the exception already indicated, the date of the last menstrual period was known, the day of the cycle to which the specimen corresponded was determined. The specimens were obtained from cases of uterine fibromyomata, adnexal inflammation and ovarian cysts. It may be argued that as this material was pathological it was valueless for determining physiological relationships, and this argument must be considered. In the first place it is unlikely that any accurate investigations will be carried out on normal human ovaries, for post-mortem material is almost useless as no accurate menstrual history is available and post-mortem changes prevent the recognition of fine histological characters. Secondly, the alternative method of removing corpora lutea from healthy ovaries during abdominal operations is inaccurate, for not only is it quite

common to mistake blood cysts and follicular cysts for corpora lutea but it is never certain that the corpus luteum removed is the youngest present in the two ovaries. Although pathological conditions were present in the cases I examined, the menstrual cycle was regular and there was no reason to believe that the factors controlling menstruation were abnormal.

The method of investigation was as follows. Both ovaries were examined, each ovary being divided into a series of slices of about 5 mm. thickness. Frozen sections were made from any which looked promising and then the whole series was embedded in paraffin. Sections were made from each block and examined. If no corpus luteum was found, each slice was cut down by 0.5 mm. and more sections made. This series of sections was then examined and the process repeated until the whole of the material had been used. In this way about 100 sections were made from each ovary and a complete examination of both ovaries with respect to young corpora lutea was obtained. The great advantage of this method was that the youngest corpus luteum present was found microscopically, and not with the naked eye. Further, young corpora lutea were not likely to be missed with the technique I followed; and, lastly, at the conclusion of the investigation, the physiological state with respect to ripening follicles and corpora lutea was established.

An enormous amount of work has been done on the development of the corpus luteum to decide whether the lutein cells are derived from the granulosa or from the theca interna. The modern view is that the granulosa cells are responsible and recent researches have been undertaken to decide the fate of the cells of the theca interna layer. My specimens show without doubt that the large lutein cells of the corpus luteum are derived from the granulosa layer.

#### *Proliferation and degeneration of the corpus luteum.*

Of the series of 22 cases of patients who were menstruating regularly, four possessed a proliferating corpus luteum in the ovaries. In three of these four cases accurate menstrual histories were obtained. These specimens corresponded to the 17th, 18th and 19th days. In the fourth case, although the patient was menstruating regularly, she was uncertain of the date of her last period, but from the history given it appeared that the specimen corresponded to somewhere between the 14th and 17th days. This specimen is the earliest example of a corpus luteum I have found. As in each of these four cases the corpus luteum



was proliferating it follows that ovulation must have occurred before and probably not long before the 17th day.

Three cases corresponded to the phase between the 19th and the 27th days. In each the corpus luteum was mature. The cells of the lutein layer were large and polyhedral and their nuclei showed no mitoses. The protoplasm was faintly granular, taking on an old gold colour with van Giesen's stain and with paraffin imbedded sections no vacuolations were found. No hyaline tissue was deposited within the cavity, or between the lutein cells, or at the periphery between the lutein layer and the theca interna. The theca interna cells could be observed at the periphery forming a thin ring surrounding the lutein layer and filling the invaginations produced by the convolutions of the latter, much smaller than the lutein cells and differing from them in their staining properties. With frozen sections stained with fat stains it was found that the lutein cells contained no fat, while the theca interna cells gave the reactions of neutral fat. Consequently, Scharlach R. and Sudan III serve as excellent differential stains for the two layers. Coruer(12), and Solounous and Gatenby(13), have shown that osmium tetroxide also brings out this differentiation. At this stage the cavity of the corpus luteum contains very little blood clot. It is filled with serous fluid but a few strands of fibrin can be seen. I would point out here that at this phase of the cycle the corpus luteum is mature while in the uterus the endometrium displays the pre-menstrual changes.

One case corresponded to the 28th day—the last day of the cycle, and five cases corresponded to the first five days of the cycle—that is to say, to the period of menstrual bleeding. In each of these six cases the most recent corpus luteum was degenerating and showed the beginning of the formation of a corpus albicans. These degenerative changes were as follows. The old gold reaction with van Giesen's stain was lost, although traces of it could still be identified in the early specimens of this phase. The protoplasm of the lutein cells stained badly and was vacuolated. Hyaline tissue—well brought out with Mallory's stain—was deposited between the lutein cells and a thin ring of hyaline tissue appeared within the cavity adjacent to the lutein layer. With frozen sections it was found that the lutein cells contained fat in the form of neutral fat and probably this fat accounted for the vacuolations seen with paraffin imbedded sections. The theca-lutein cells were still identified and at this stage contained fatty acids; consequently Nile blue sulphate staining gave a beautiful differentiation. Most of the specimens corresponding to this period contained large amounts of blood in the cavity.

Nine cases corresponded to the phase between the 6th and 13th days of the cycle. In each of these cases the most recent corpus luteum was degenerate and to a more marked degree. In none was a young corpus luteum found, though ripening follicles were seen in plenty. The degeneration continued along the lines described above, the main feature being the deposit of hyaline tissue within the cavity and between the lutein cells. In this way the corpus luteum is gradually converted into a corpus albicans.

No specimen corresponding to the phase between the 13th and 17th days was obtained beyond the unreliable one already mentioned.

### *Discussion.*

The interpretation of the results may now be considered. Ovulation must occur before the 17th day for specimens of the most recent corpora lutea in the ovaries of cases corresponding to the phase between the 17th and 27th days were either proliferating or mature. On the other hand, ovulation cannot occur before the 13th day, for with the technique I followed young corpora lutea would have been observed if ovulation occurred before this day. Consequently ovulation must occur between the 13th and 17th days—most probably about the 14th day.

The second problem is that of the function of the corpus luteum. I have shown that a corpus luteum attains its state of maturity about ten days before the onset of menstruation, that just before menstruation begins it commences to degenerate and that this degeneration then continues until a corpus albicans is produced. On the other hand, if pregnancy occurs the corpus luteum persists. The corpora lutea of 12 cases of ectopic gestation were examined. These specimens corresponded to the early weeks of pregnancy and it was found that in the early specimens—up to about 12 weeks—the corpus luteum of pregnancy resembled very closely the mature corpus luteum of the latter half of the menstrual cycle. The general form of the lutein layer is almost identical though because the lutein cells are larger, the convolutions have become more intricate and in this way isolated nests of lutein cells are frequently found in the cavity when sections are examined. The lutein cells stain well, are not vacuolated and contain no fat. There is no deposit of hyaline tissue within the cavity or between the cells of the lutein layer. The theca interna cells are seen in the same situations that they occupy in the mature corpus luteum of the menstrual cycle. The close resemblance of the corpus luteum of early pregnancy to this latter structure is therefore apparent.

Minor differences have been described by Marcotty<sup>(14)</sup> and Miller<sup>(15)</sup> but they are not very apparent nor have they any significance

To sum up ovulation occurs between the 13th and 17th days of the cycle, a corpus luteum is formed which rapidly attains maturity and which degenerates just before the beginning of the next menstrual period. If the woman becomes pregnant, the period is missed and a mature corpus luteum is found in the ovaries which closely resembles the mature corpus luteum found during the latter half of the menstrual cycle. This suggests that if fertilisation ensues the corpus luteum does not degenerate but persists. In the case of the endometrium, activity is first seen about the 11th day. Pre menstrual changes rapidly develop and immediately before menstruation the endometrium resembles the decidua of early pregnancy. During menstruation degeneration of the pre-menstrual endometrium occurs and the greater part of it is shed. Repair occurs during the early part of the post menstrual phase and the whole process is then repeated in the next cycle. If pregnancy ensues the period is missed and the pre-menstrual changes are developed in higher degree to form the decidua. Consequently, simultaneously with the presence of a mature corpus luteum in the ovaries, a pre menstrual endometrium exists in the uterus. When this corpus luteum degenerates in the ovary, the pre-menstrual endometrium degenerates in the uterus and menstruation occurs. During early pregnancy a mature well developed corpus luteum is found in the ovaries and the decidua is found in the uterus. It is therefore reasonable to assume, since the ovaries control menstruation, that the function of the corpus luteum is to produce the pre-menstrual changes which, if pregnancy occurs, becomes better marked in the decidua.

So far the evidence in support of this view has been histological and perhaps the experimental evidence is best considered from the historical aspect. Foremost amongst the early workers on the corpus luteum stands Ludwig Fraenkel. Inspired by his former teacher, Gustave Born, Fraenkel carried out his classical researches on rabbits. Born had suggested from the consideration of the incidence of uterine decidua in cases of ectopic gestation, that the corpus luteum controlled the implantation of the fertilised ovum. Fraenkel's experiments were carried out on rabbits, animals in which ovulation is known to occur about ten hours after copulation, so that the investigations could be accurately timed. Fraenkel showed that if the corpus luteum was removed from the impregnated rabbit the fertilised ovum either was not imbedded or was aborted early in pregnancy. The experiments were accurately

controlled and afford ample proof that the implantation of the fertilised ovum is controlled by the corpus luteum. The mechanism by which this is achieved, however, was not adequately explained.

In the case of human beings there was very little reliable work to indicate the phase of the menstrual cycle when ovulation occurs, until the investigations of Hitschmann and Adler indicated that the pre-menstrual changes in the human endometrium were produced to prepare the endometrium for the imbedding of the fertilised ovum. The corollary of this view was that ovulation probably occurred in the inter-menstrual phase. On the other hand, there was a certain amount of clinical evidence to indicate that ovulation occurred either during menstruation or in the post-menstrual phase. Further, it was suggested by Heape that menstruation represented the pro-œstrous phase of lower animals (this view being adopted by Marshall who afterwards modified it) and as in certain animals ovulation is known to take place during the post-œstrous period, the view that ovulation occurs in the inter-menstrual phase was disputed. With human beings the date of occurrence of ovulation can only be determined by obtaining accurately-timed specimens of young corpora lutea. This achievement was made by R. Meyer(10), R. Meyer and Carl Ruge(16), and afterwards by Schröder(17), and by Novak(11), and all agreed that with human beings ovulation occurs in the inter-menstrual phase between the 10th and 17th days. Again, the view of Marshall has been modified since the work of Keller(18), and Marshall and Halnan(19), who have shown that in the post-œstrous phase changes simulating the pre-menstrual characters of the endometrium can be observed, thus disproving the conception of the parallel between pro-œstrus and menstruation.

In *An Introduction to Sexual Physiology* just published, Marshall has given his present views clearly suggesting that the destructive stage of the endometrium finds its parallel in lower animals to pseudo-pregnant and pro-œstrous degeneration telescoped into one. Again, Corner(20) has shown that in *Macacus rhesus*, an animal with a menstrual cycle of about 27 days, ovulation takes place about 12 to 14 days before the onset of the menstrual flow.

The next question to consider is the relation between the corpus luteum and the imbedding of the fertilised ovum. It was shown by Leo Loeb(21), that a decidual reaction to local mechanical stimuli occurs in the endometrium provided that the ovaries are functioning normally, but that if the latter are removed this reaction no longer occurs. Ancel and Bouin(22), showed that if in rabbits the buck is rendered sterile by

## OVARIES AND MENSTRUATION.

ligation of the vasa deficientia ovulation in the female occurs not after copulation and a corpus luteum is formed, and further the proliferative changes follow in the endometrium. Again, in the ectopic gestation in human beings, it is well known that the endometrium of the uterus becomes converted into the decidua. These considerations suggest very strongly that the corpus luteum in the female is responsible for the production of the pre-menstrual endometrium of the decidua if pregnancy supervenes, and this conception of the explanation of the non-implantation or abortion of the fertilised ovum if the corpus luteum is removed. These deductions from the experimental work agree with the conclusions obtained from the histological observations described above.

Fraenkel had stated even before the work of R. Meyer, that in human beings ovulation probably occurred in the inter-menstrual period basing his opinion upon the macroscopical examination of ovaries after abdominal operations. He attempted to show that a parallel existed between his experiments on animals and the result of excision of the corpus luteum in human beings. He stated that if the corpus luteum was removed in the latter half of the menstrual cycle menstruation was delayed for three to four weeks. These results were criticised severely by Halban, and Halban and Kohler<sup>(23)</sup> published a series of cases and showed that if the corpus luteum was removed in the latter half of the menstrual cycle menstruation occurred prematurely within about 18 hours after the operation. These experiments were confirmed by G. Cotte<sup>(24)</sup>, and I have the records of three cases in which the corpus luteum or the ovary containing the corpus luteum was removed in the pre-menstrual phase and where menstruation took place 36 hours after the operation in each case. On the other hand, in a case where the ovaries were removed in the post-menstrual phase a uterine hæmorrhage followed. Further, Halban and Kohler state that if the corpus luteum after removal was grafted beneath the anterior abdominal wall menstruation did not occur prematurely but took place at the expected time. Although it is well known clinically that uterine hæmorrhage frequently takes place after minor operations on the tubes and ovaries without removal of the corpus luteum, the latter experiments of Halban and Kohler indicate that when the corpus luteum is present in its state of maturity menstruation does occur. The interpretation of these experiments can only be that the presence of the corpus luteum is essential for the existence of the pre-menstrual changes in the endometrium and that if the corpus luteum

is removed in its state of maturity menstruation occurs. This is in agreement with the view put forward from the histological investigation. Degeneration is always to be seen in the corpus luteum during menstruation and has been observed in a specimen corresponding to the last day of the menstrual cycle. During menstruation a degeneration of the endometrium takes place with shedding of the superficial zones of the pre-menstrual endometrium.

In this way a simple solution of the relation between ovarian function and menstruation is obtained. But no explanation of the incidence of the degeneration of the corpus luteum immediately before menstruation has been given. It was suggested by R. Meyer that the ovum itself controlled the development of the corpus luteum, that if fertilisation occurred the corpus luteum persisted: but that if the ovum died the corpus luteum degenerated and menstruation occurred. This attractive hypothesis has much in its favour but it cannot be entered into here without a full discussion of the processes of follicular ripening and atresia.

#### CONCLUSIONS.

1. The pre-menstrual changes are first observed about the 14th day of the cycle, the cycle being regarded as commencing on the first day of the menstrual discharge. At the end of the menstrual cycle the pre-menstrual endometrium resembles the decidua of early pregnancy.

2. During menstruation a degeneration of the hyperplastic endometrium occurs which results in the shedding of the superficial portions of the latter.

3. Ovulation in the human female takes place between the 13th and 17th days of the cycle.

4. The corpus luteum attains its state of maturity on the 19th day and persists in its mature form until about the 27th day.

5. On the 28th day and during menstruation the corpus luteum is degenerate.

6. If pregnancy occurs the corpus luteum persists in its state of maturity until about the 12th week and the pre-menstrual changes of the endometrium become better developed to form the decidua.

7. Evidence has been brought forward to suggest that the corpus luteum is responsible for the production of the pre-menstrual changes and of the decidua if pregnancy occurs.

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# THE RELATION BETWEEN THE SIZE OF THE HEART AND THE OXYGEN CONTENT OF THE ARTERIAL BLOOD. BY K. TAKEUCHI.

*(From the Physiological Laboratory, Cambridge.)*

THE object of the following research was to correlate the degree of dilatation of the heart with the oxygen content of the arterial blood. It is, of course, currently known that a sufficient degree of anoxæmia produces cardiac dilatation, yet out of five subjects<sup>(1)</sup> who were investigated at an altitude of over 14,000 feet in Peru, and who undoubtedly had an abnormally low oxygen content in the arterial blood, none showed any sign of a larger X-ray heart shadow whilst in three cases the shadow was actually smaller than at the sea level. There seemed to be two possible explanations of the fact that the heart in these cases did not dilate, (1) that the degree of anoxæmia was insufficient, (2) that acclimatisation to the anoxæmia had taken place. I have, then, investigated the immediate effect of anoxæmia and whether some critical degree of anoxæmia is required before dilatation of the heart takes place. The experiments were made on cats anæsthetised with urethane and C.E.

At first some crude experiments were made in which the chest was opened and the heart viewed through a glass plate. The general impression of the size of the heart could be sketched on the plate with a grease pencil and even this simple and inaccurate process left no doubt that when the oxygen in the air breathed by the cat was reduced the area traced on the glass immediately became larger. This method was unsatisfactory because (among other reasons) it gave no information as to whether the alteration of the size of the heart was principally an alteration in the systolic or in the diastolic size, and, indeed, X-ray photos are none too definite on this point. Prof. Langley suggested that we should use the cinematograph and after a number of preliminary experiments, three were obtained which are worth recording.

The general plan of these experiments was that the chest was opened, artificial respiration maintained, and the inspired air varied in composition. A preliminary investigation of the conditions obtaining in the open chest, when the animal was under artificial respiration, was undertaken. A description of the apparatus ultimately used and a statement of its efficiency are recorded in another paper<sup>(2)</sup>.

The experiment was divided into four or five periods, the com-



position of the inspired air being gradually changed. In the first period a mixture of oxygen and atmospheric air was given; in the second, air; in the third, a mixture of air and nitrogen, and in the fourth a mixture richer in nitrogen than the third. During each period a short film was taken. The pressure of inspired air and the rate of rotation of the motor of the artificial apparatus were kept constant so that the respiratory conditions, other than the composition of the air remained constant. The  $\text{CO}_2$  was therefore removed at the same rate as in an experiment in which air was breathed the whole time. The programme for a single experiment was as follows.

Two samples of each of expired and inspired air were collected in a football bag and Barcroft's tonometer respectively at each period of the experiment,  $\text{O}_2$  and  $\text{CO}_2$  in the samples were measured by Haldane's apparatus and the total volume of expired air per min was measured by gas meter. Two samples of arterial blood were taken from the carotid artery at each period and the percentage saturation of oxygen was measured by Barcroft's differential manometer. All the samples and the photographs were taken as nearly as possible at the same time so as to get simultaneous results. The cinematograph photographs taken of the heart beating at each period, were enlarged and measured in length, width and area of hearts



Fig. 1.

To obtain a record of the horizontal projection of the area prints were taken of the films. The photographs of the heart were cut out and traced on to millimetre squared paper. The number of square millimetres which the figure covered was then counted.

Three such experiments were performed. Fig. 1, which reads horizontally, shows a typical record of the successive photos cut out from five strips of film; each strip corresponds to one period of the experiment, the first period being at the top. The following are the data:

*Exp. 3. Cat under urethane.*

Time		
	0 m.	Start operation.
1 h.	12	Finish operation.
"	24	Period I commences (oxygen).
"	29-35	Samples of inspired and expired air taken.
"	36	Arterial blood.
"	38	Film I.
"	45	Period II commences (air).
"	48-52	Samples of expired and inspired air.
"	53	Samples of arterial blood.
2 h.	2	Film II.
"	2 $\frac{1}{2}$	Period III commences (nitrogen let into mixing bottle).
"	4-5	Samples of inspired and expired air.
"	4	Film III.
"	9	Period IV, atmospheric air.
"	11-15	Samples and inspired and expired air.
"	15	Arterial blood.
"	21	Period V, nitrogen.
"	21	Film V.

Period	Inspired air p.c. of O <sub>2</sub>	Analyses. Expired air		Arterial blood p.c. sat. with O <sub>2</sub>	Comparative area of heart
		p.c. O <sub>2</sub>	p.c. CO <sub>2</sub>		
I	93	92	1.2	100	106-94
II	21	19.5	1.2	93	111-106
III	15.5	13	7.9	7	164-152
IV	21	19.5	1.3	93	115-103
V	Low	—	—	Low	126-103

Each change in the composition of the air breathed produces an immediate alteration in the size of the heart. The rapidity of the response is a matter which is as remarkable as the degree. Thus, only 2 minutes elapsed between films II and III. Yet even without the aid of measurements the difference is obvious enough in the figure.

Further, the alteration appears to be reversible for in period IV the heart has reverted to the condition of period II. In the table of analyses the largest and smallest areas of the heart in each film are given and the two periods (IV and II) correspond very closely.

A third point to be observed is that there is a definite dilatation of the heart in period I as compared with period II.

Unfortunately it was impossible to get blood samples for period V which purports to be a repetition of period III. The film of period V is however worth reproducing, because as compared with that of period III, there is a much greater difference between systole and diastole. Possibly dilatation affects the diastole before the systole, but that is a matter for future investigation. The phenomenon is shown very clearly in Fig. 2.

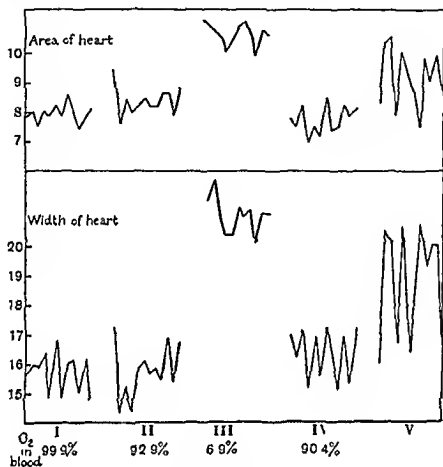


Fig. 2

in which the dimensions of the heart are plotted in relative terms. It must not be supposed that the amplitude of the heart-beat is as irregular as the oscillation on the diagram. The figures represent really a series of interference observations affected equally by the frequency of the heart-beat and the frequency of the exposure of successive films, but it may be taken that the maximal and minimal measurements, approximately represent the diastole and systole respectively.

A comparison of the amplitudes for width and area in Fig. 2 show that the two vary in strict relationship to one another. The variations in the amplitude of the length are much more constant and also smaller.

Amplitude expressed as percentages of the minimum

Period	I	II	III	IV	V
Area	15	24	15	19	32
Width	12	19	12	13	30
Length	7	6	7	5	8

But although the variations in length are rather constant for each heart-beat whether the heart be dilated or not, the length takes part in the general dilatation due to anoxoemia as may be seen from the figure. The length measurements are much more difficult to be certain about than those of the width.

In Exps. 1 and 2 the routine was a little different. Instead of an alternation between air and an oxygen-poor atmosphere with two periods of each (periods II-V), there was a gradual transition from oxygen to the atmosphere richest in nitrogen over the first four periods, whilst the fifth was air. The general result was quite in accordance with Exp. 3, there being only one apparent discrepancy, namely, that administration of air at the end of Exp. 1 did not reduce the heart. The reason was that the air came too late, the animal being by that time moribund and the air did not prevent its dying. Probably the circulation had almost ceased though the heart was just beating visibly when the air was given.

Fig. 3 represents a composite picture of the relation of the percentage

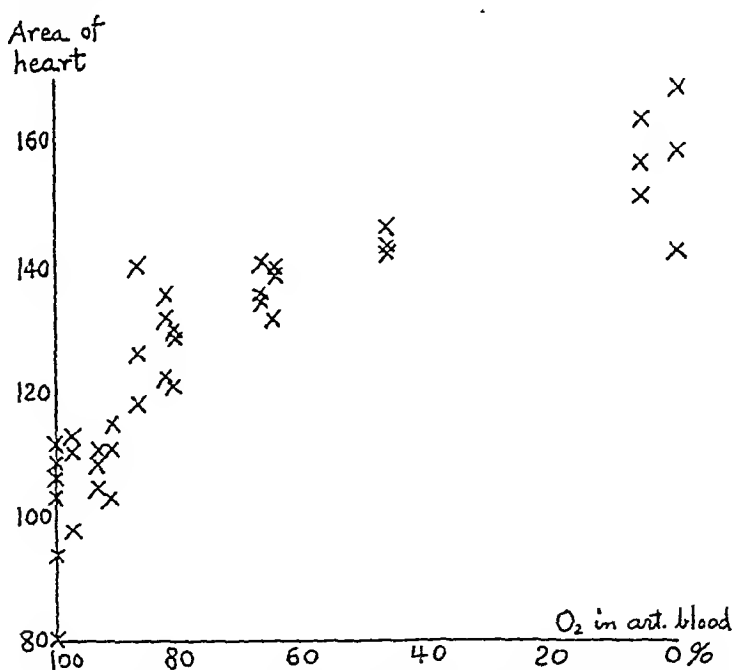


Fig. 3. Relation between area of heart and percentage saturation of oxygen in arterial blood.

saturation of the arterial blood to the limits of area of the heart shadow. It gives the definite impression that the most considerable changes in

dilatation are in the earlier stages of anoxæmia. The average saturation of the blood of the person observed in Peru was about 85 p.c. and similar saturations have been observed by Adair and Gildea (1, p. 362) in the Rocky Mountains. The trend of the present research is to show that at 85 p.c. saturation the heart is considerably dilated.

Clearly results brought about by rapid changes in the cat cannot be compared unreservedly to alterations which are brought about by gradual changes in man. Apart from the difference of the species of animal used, the time factor must be considered and the suggestion is that when fit men live at high altitudes acclimatisation takes place. How far the acclimatisation lags after the dilatation we do not know, it may be that the acclimatisation can take place almost as fast as the person ascends, so that the dilatation is only potential, but the single observation made on this subject by the Pern party did seem to show that Makin's heart became reduced in size between the time of his arrival at Oroya (12,000 feet) and that of his subsequent departure from Cerro (14,000 feet). Again, we have noted that in our experiments the reduction of the heart area on the transition from an oxygen poor atmosphere to air is immediate. It must not be supposed that the immediate administration of oxygen to a patient who has been subject to anoxic conditions will necessarily have the same effect. Somervell observed not only that on Mount Everest the cardiac dilatation was the rule, but that the dilatation did not go away for some time after the party came down, in fact till they reached Darjeeling. It would appear likely that the rapidity with which oxygen reduces a heart which has been dilated owing to anoxæmia, depends on the length of time over which it has been dilated. In these experiments the dilatation though extreme, was for only a few minutes and it was abolished immediately on giving oxygen. In the case of the Everest party, the dilatation lasted over weeks and took a correspondingly long time to pass off.

*The work done by the heart.* It scarcely needs pointing out that in the anoxæmic periods the heart is working much less economically than in the normal ones. In accordance with the views put forward with regard to striated muscle by A. V. Hill (3) and applied to the heart by Stalling (4), the energy expended at each beat is a function of the length of the fibre. It has been shown by Doi, that in preparations such as we used the total output of blood per minute is not increased. It is not possible to make any calculation from the figures given above of the energy expended by the heart for a given output of blood. We know from the work of Doi that the output of blood will fall off, we have

the pulse rates, and the maximal width or length in each period to give some idea of the degree of elongation of the fibres. Thus in Exp. 3.

Period	Breathing	Pulse rate	Relative width
II	Air	125	17
III	Nitrogen + air	105	22
IV	Air	125	17

From the above it appears that in this experiment the heart slows almost exactly in the same proportion as it dilates. If one regarded the potential energy liberated per minute as

$$\frac{1}{6} TLF,$$

where  $T$  is the maximal tension,  $L$  the length of the fibre in diastole, and  $F$  the pulse rate, and taking the change of diameter as a measure of  $L$ ,  $LF$  remains nearly constant and the potential energy would vary roughly with the systolic pressure. Here, again, the conditions differ from those of a person at great heights when the pulse is dilated and *quicken*ed without apparently any increased output as the result.

### SUMMARY.

1. The reduction of oxygen in the air breathed by a cat produces immediate enlargement of the heart. The re-administration produces immediate reduction.

2. The principal effect on the size of the heart seems to be in the less extreme ranges of anoxœmia, thus the alteration is much greater between 100 p.c. and 80 p.c. saturation of the arterial blood than between 40 p.c. and 20 p.c.

3. The greater elongation of the fibres means a greater output of energy at each beat, other things being equal.

4. The time relations given in this paper cannot be applied to conditions of anoxic anoxœmia of long duration without reference to the degree of compensation of the chronic changes set up in the heart.

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## THE DOUBLE INNERVATION OF THE M. GASTROCNEMIUS. BY S DE BOER

*(From the Pathological Laboratory of the University of Amsterdam)*

GAD(1) was the first who examined the plurisegmental innervation of the gastrocnemius of the frog. With a single induction shock he stimulated separately both anterior roots which innervate the gastrocnemius muscle. Afterwards he stimulated the two roots simultaneously. The result was that the tension which developed in the gastrocnemius after simultaneous stimulation of both anterior roots was equal to the sum of the tensions caused by stimulation of the separate roots. He obtained the same result when he stimulated tetanically.

EXNER(2) found other relations with the M. cricothyreoideus of the rabbit. It is innervated by the N. laryngeus superior and the N. laryngeus medius. After cutting one of these nerves, EXNER found no degeneration of the muscle fibres and he concluded that in this case each muscle fibre was doubly innervated. His pupils, LEDERER and FRIEDA LEMHERGER(3), controlled this result by using the tension indicator of FICK. They found that the tension on simultaneous stimulation of both nerves was equal to the sum of the tensions on excitation of the nerves separately. This result was against the double innervation supposed by EXNER. As regards the Mm. flexores digitorum communis profundus and sublimus, which these investigators examined by the same method, they came to the conclusion that all, or nearly all muscle fibres have a double innervation. AGDUHR(4) cut at different times two of the anterior roots which innervate the M. flexor digitorum communis sublimus of the cat. In this way he found in the same muscle fibre different end plates, which were connected with nerve fibres arising from different segments. Opinions about the innervation of the M. gastrocnemius still differ. F. W. FRÖHLICH(5) as well as GAD, supposes a separate innervation of the several muscle fibres. BERITOFF(6), however, holds that most of the muscle fibres have a double innervation. When he stimulated one of the anterior roots tetanically he did not obtain any increase of the muscle curve by exciting the other root. In 1921 two communications appeared on the innervation of the gastrocnemius. SAMOJLOFF(7) registered the action-currents of the gastrocnemius after

simultaneous and separate stimulation of the two anterior roots supplying the muscle. He found that the curve of the action currents obtained after simultaneous stimulation of the two roots was equal to the sum of both curves, obtained by stimulating them separately. As Samojloff, however, used biphasic leading off, the experiments afford no proof of the single innervation of the muscle fibres. In a following series of experiments Samojloff first fatigued the muscle after stimulation of one anterior root. After that he stimulated the other anterior root intermittently until there was fatigue on this side also. When he then stimulated intermittently the first anterior root he found signs of recovery. Based on these results Samojloff concluded that each muscle fibre is innervated only from one anterior root. In the same year Cattell and Stiles(8) came again to the opposite opinion, that is to say, that the greater part of the muscle fibres of the gastrocnemius receives nerve fibres from two spinal segments. They found that the tension provoked by the muscle if each of the two anterior roots is stimulated, is about as great as when both roots are stimulated. If by means of rhythmical stimulation of one anterior root fatigue is produced, stimulation of the other anterior root gives a normal effect and a second fatigue can be produced by it comparable with the first. The explanation of this they take to be that the fatigue does not occur in the contracting mechanism but in the end plates of the nerves.

Previous investigators have used the M. gastrocnemius removed from the body. I have now studied the question by a new method leaving the muscle *in situ* and with the circulation of the blood more or less intact.

*Method.* The spinal cord was severed with a pin a little below the skull. The abdomen was opened and the 8th and 9th nerves (numeration of Langley and Orbeli(9)) were tied and cut near their exit from the vertebræ. Frogs in which the 7th nerve caused contraction of the gastrocnemius were discarded. The nerves were placed on separate electrodes and covered with moist cotton wool. The thigh was firmly pinned down and the tendon of the gastrocnemius connected with a recording lever. Then five to eight drops of acetate of veratrin 1 p.c. were injected into the dorsal lymph sac. When stimulation of one of the nerves caused a veratrin contraction—which was in about a quarter of an hour—the experiment began. As is known, the increased contraction caused by veratrin is abolished when the stimuli are repeated at short intervals. The plan of the experiments was to stimulate one nerve until the veratrin effect had disappeared and then to stimulate the other nerve. The



stimulation was by break shocks sent in at the rate of one or two a second. By means of a commutator in the secondary circuit of the induction apparatus the stimuli could be rapidly transferred from one nerve to the other.

Fig. 1 shows the result of an experiment. The top line gives the contraction of the gastrocnemius after the 9th nerve had been stimulated every 2 seconds during 10 minutes. Then the 8th nerve received a

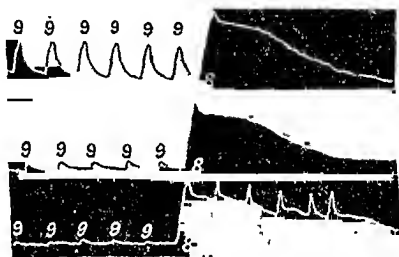


Fig. 1.

induction shock. It will be seen that each stimulation of the 9th nerve caused a single twitch with signs of fatigue whilst stimulation of the 8th nerve gave rise to a distinct veratrin curve. The second line gives the twitches after the 9th nerve had been stimulated every 2 seconds during 20 minutes. The single excitation of nerve 8 shows a complete veratrin curve. The same effect showed itself after nerve 9 had been stimulated for half an hour. A stimulus then applied to the 8th nerve caused a veratrin curve (see the bottom line, Fig. 1; here nerve 8 was stimulated several times during the contracture).

In another experiment, after obtaining a veratrin curve on stimulation of each nerve, I applied to the 8th nerve an induction shock every second for half an hour. In Fig. 2 the small twitches then obtained are shown. An induction shock then applied to nerve 9, causes a veratrin curve. Nerve 9 was stimulated every 2 seconds during half an hour. In this half-hour the veratrin effect of nerve 8 recovers entirely, as we see on the bottom line of Fig. 2.

We could imagine that after some excitations, the contraction disappears from the veratrin curve because the end plates of the nerve cease to conduct the excitation for the contracture. I have, however, excluded this possibility. I stimulated the sciatic nerve until it caused

a single twitch, then I stimulated the gastrocnemius directly; this also caused a single twitch. Direct stimulation of the muscle never caused a

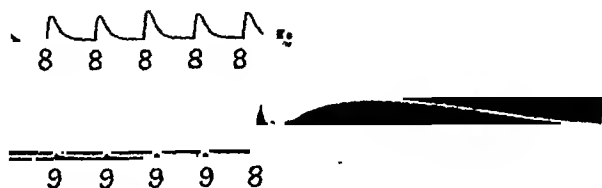


Fig. 2.

veratrin contraction, if by rhythmical stimulation of the sciatic the veratrin contraction had disappeared.

From this I conclude that the veratrin contractions of Figs. 1 and 2 arose in different muscle fibres from those giving the immediately preceding twitches and therefore that the two nerves innervate separate parts of the gastrocnemius.

When Samojloff(7) noticed fatigue after rhythmical stimulation of the one root, he stimulated the other root rhythmically and obtained some but only a slight recovery on then stimulating the first root. As Samojloff worked with excised muscles I expected to get a better result with my preparations in which the circulation of the blood continued. The 8th and 9th nerves were stimulated alternately once each second for 3 minutes at a time. After fatigue had been produced, the contractions produced by each nerve at the beginning of its period of stimulation were always markedly greater than those produced at the end of the last period of stimulation of the same nerve. Fig. 3 illustrates the results. The upper line shows the contractions obtained from the 9th nerve at the end of the fifth period of its stimulation, and those obtained (at 8 in the figure) at the beginning of the sixth period of stimulation of the 8th nerve. The lower line shows the contractions obtained at the end of the sixth period of stimulating the 8th nerve, and those obtained on switching the current on to the 9th nerve (at 9 in the figure). It is evident that two parts of the muscle work entirely independently of each

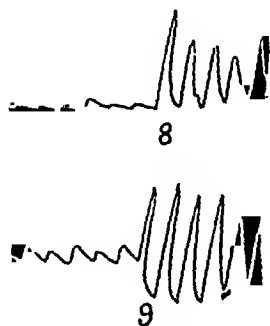


Fig. 3

other and are excited independently from two different spinal segments. I leave open the possibility that some of the muscle fibres may receive nerve fibres from both segments. We must conclude from these experiments that the muscle fibres are insulated in the electrical sense, for the action currents which arise in contracting fibres do not stimulate the fibres which remain quiet. Thus, in muscle fibres, the relation is not the same as that in the well known experiment of the secondary twitch of Mattencei. In a chemical sense also the muscle fibres are insulated, as Samojloff concludes from his experiments on fatigue. As we have seen, the disappearance of a veratrin contraction in one set of the muscle fibres does not lessen the veratrin contraction of the other set. Lamm(10) thinks that after many stimuli, the veratrin effect disappears because the action of the veratrin is disturbed by lactic acid. If this assumption is right then the lactic acid does not diffuse to the other fibres, not even after half an hour's stimulation. From the second series of experiments also it is evident that the two parts of the muscle work independently of each other in a chemical sense. The part of the muscle which was fatigued recovers whilst another part becomes exhausted. The metabolic products under influence of which the exhaustion arises do not diffuse to the neighbouring inactive muscle fibres.

#### SUMMARY.

1. The gastrocnemius muscle of the frog, as is known, contracts when either the 8th or the 9th spinal nerve is stimulated. If the frog is poisoned with veratrin and one of these nerves is stimulated with successive shocks until the veratrin form of contraction disappears, stimulation of the other nerve will give a typical veratrin contraction.

2. If in a frog in which the blood circulation is maintained, either spinal nerve is stimulated rhythmically until the contractions are those of great fatigue and the other nerve is then stimulated, the contraction caused by it shows no sign of fatigue. Further recovery from the fatigue which has been produced by one nerve is unaffected by fatigue stimulation of the other nerve.

3. Thus the 8th and 9th spinal segment innervate different sets of muscle fibres in the gastrocnemius muscle. The experiments, however, do not show that no muscle fibre has nerve endings from two segments.

4. Fatigue products do not diffuse from one set of fibres to the other, nor do the action currents accompanying the contraction of one set stimulate the fibres of the other set, so that the two sets may be considered as chemically and electrically insulated.

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ON HELICORUBIN AND ITS RELATION TO  
HÆMOGLOBIN<sup>1</sup>. BY M L ANSON AND  
A E MIRSKY (*National Research Fellow*)

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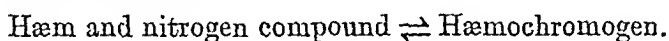
WE have recently shown that ordinary hæmochromogen, as well as hæmoglobin, is a conjugated protein. It consists of hæm (an iron pyrrol complex) and globin. Hæmochromogen can be changed into hæmoglobin merely by changing the [H] of the medium. If an alkaline solution of hæmochromogen is neutralised, the hæmochromogen turns into hæmoglobin. Since the molecular weight of hæmochromogen is about 17,000 and that of hæmoglobin about 67,000, hæmoglobin appears to be a polymer of four molecules of hæmochromogen. Globin-hæmochromogen is only one of many hæmochromogens which either exist in nature or can be made in the laboratory. A hæmochromogen consists of hæm joined to a nitrogen compound (protein, amino acid, amine, ammonia, pyridine, etc.). The differences in hæmochromogens are due to the differences in their nitrogen compounds for the hæms of all known hæmochromogens are identical<sup>(2)</sup>.

With these views on the nature of hæmochromogen and hæmoglobin we considered the properties of hæmoglobin—its specificity, solubility, its ability to combine loosely with O<sub>2</sub> and CO, and the temperature coefficient of this reaction, its increased acidity on combining with CO or O<sub>2</sub>, etc. We attempted to determine which of them are due to hæm, which to the influence of a nitrogen compound on hæm (as in a hæmochromogen), which to the influence of globin in particular (as in globin-hæmochromogen) and which to the polymerisation of hæmochromogen to form hæmoglobin. For example, the specificity of hæmoglobin, as shown by its affinity for a gas, was found to be dependent on polymerisation, rabbit and sheep hæmochromogens, though containing different globins, have the same affinity for CO, whereas rabbit and sheep hæmoglobins have very different affinities for this gas. On the other hand, the greater solubility of hæmoglobin, as compared with hæm, is due to all the possible factors—its possessing a nitrogen compound,

<sup>1</sup> [This work on helicorubin is based on a theory of the hæmoglobin molecule which has been presented in detail elsewhere.]

its possessing globin in particular, and to its being polymerised. In other words, reduced hæm is only slightly soluble, any hæmochromogen is more soluble, globin-hæmochromogen is more soluble than any artificial hæmochromogen, and polymerised globin-hæmochromogen (hæmoglobin) is much more soluble than globin-hæmochromogen itself.

When it came to the question of what makes oxyhæmoglobin a stronger acid than reduced hæmoglobin we ran into experimental difficulties. We wanted to compare the effects of  $[H]$  on the affinities of reduced hæm and the hæmochromogens with that of hæmoglobin for CO or O<sub>2</sub>. It was possible to obtain the CO dissociation curve of globin-hæmochromogen in very alkaline solution—so alkaline that any acid groups of the hæmochromogen must have been completely ionised—but it was impossible to measure the affinity of globin-hæmochromogen at any  $[H]$  at which its acid groups would be either only partly ionised or unionised. To make these measurements it is necessary to obtain the substances in somewhat more than insignificant concentrations. At any but the very alkaline  $[H]$ 's reduced hæm and the hæmochromogens are either too insoluble or unstable. In approximately neutral solutions these substances are almost completely insoluble. In acid solutions reduced hæm is completely insoluble. Globin-hæmochromogen appears to be unstable in acid medium. In any hæmochromogen system there is an equilibrium of this nature:



In alkaline medium, globin has such a great affinity for hæm, that the equilibrium is shifted almost entirely to the right. In acid medium, however, the equilibrium is shifted to the left, and practically no hæmochromogen is present. Instead there is a mixture of reduced hæm and globin; the former soon precipitates. No artificial hæmochromogen could be used instead because no other nitrogen compound seems to have nearly as great an affinity as globin for hæm. It looked as if unsuitable experimental material prevented a further enquiry as to why oxyhæmoglobin is a stronger acid than reduced hæmoglobin. Helicorubin provided the proper material for this investigation and also proved to be an interesting respiratory pigment in itself.

*History of helicorubin.* Helicorubin was discovered in 1876 by Sorby in the common large snail, *Helix aspersa*. MacMunn, Krukenberg, Dastre and Floresco, and Dhéré and Vegezzi have since contributed to our knowledge of this pigment. It occurs in the liver and gut of the pulmonate molluscs with the exception of *Planorbis*. It is

formed in the liver and poured into the gut. The only other place it is known to occur is in the liver of the crayfish. (It is interesting to note that it does not occur in the lobster.) Its absorption spectrum is similar to that of hæmochromogen, the spectrum has the same pattern, but the bands are shifted to the red, and it has therefore been called a pseudo-hæmochromogen. Its behaviour towards  $O_2$  appeared to be different from that of ordinary hæmochromogen and this was another reason for calling it a pseudo-hæmochromogen. Dhéi   and Vegezz   showed that in acid medium heliocorubin combines with  $O_2$ , but they did not enquire as to whether this combination is a true oxidation, such as the combination of  $O_2$  with globin-h  mochromogen to form hæmatin or whether it is like the loose combination between  $O_2$  and hæmoglobin in oxyh  moglobin. An alkaline solution of ordinary hæmochromogen (made from hæmoglobin) is immediately oxidised to hæmatin when exposed to the air. An alkaline solution of heliocorubin, however, remains reduced when exposed to the air. Dh  i   and Vegezz   showed that at high tensions of oxygen, oxygen would combine with alkaline heliocorubin, and thought that it did not do so readily because of reducing agents in the bile made active by the alkali. "En alcalinisant (m  me avec du carbonate de sodium) la bile, normalement acide, ces corps r  ducteurs prennent naissance et   ventuellement transforment l'oxyh  licorubin en h  licorubine alcaline." They realised that alkali might have some effect on heliocorubin, but they thought that "c'est pr  cisement surtout en cr  ant un milieu r  ducteur qu'agit l'alcalinisation." We shall show that this interpretation of the facts is incorrect and that the nature of the interaction between oxygen and heliocorubin is different from what has previously been supposed.

*Helicorubin as a hæmochromogen.* On the older views as to the nature of hæmochromogen—that it is a non-protein, iron pyrrole complex—it would be difficult to account for both the similarities and differences between hæmochromogen and the pseudo-h  mochromogen, heliocorubin. Since ordinary hæmochromogen is really a conjugated protein, and since many different hæmochromogens can be made by adding various nitrogen compounds to hæm, it seems apparent that heliocorubin may simply be a hæmochromogen with a nitrogen compound different from globin combined with the same hæm as occurs in ordinary (globin-) hæmochromogen. The way to prove that the hæms are identical would be to remove the nitrogen compounds from both globin-h  mochromogen and heliocorubin, and then to add to both hæms the same nitrogen compound. If the resulting hæmochromogens are the same, then the hæm

of globin-hæmochromogen must be the same as that of heliocorubin and the nitrogen compounds of the two hæmochromogens must be different. In this way both the resemblances and differences between ordinary hæmochromogen and heliocorubin would be accounted for. Some experiments of Dhéré and Vegezzi, which indicated that heliocorubin and globin-hæmochromogen have a common constituent, are probably of this character. Their methods were not very accurate, however. Using their spectroscope ox and sheep hæmoglobins would seem the same, whereas we now know they are different. We have substituted ammonia for the nitrogen compounds of globin-hæmochromogen and heliocorubin. The bands of the resulting hæmochromogens, when measured with the Hartridge reversion spectroscope were within experimental error ( $1 \text{ \AA}$ ) the same. The details of this experiment are described elsewhere(2).

### *Some properties of heliocorubin.*

*The affinity of its nitrogen compound for hæm.* Heliocorubin, then, is a hæmochromogen with the same hæm as that of globin-hæmochromogen, but with a very different nitrogen compound. We have shown that globin has a much greater affinity for hæm than has any other nitrogen compound we have tried. The nitrogen compound of heliocorubin, however, has even a greater affinity than has globin. If a drop of globin-hæmochromogen is placed in 50 c.c. of saturated  $\text{NH}_4\text{OH}$ , the  $\text{NH}_3$  will displace the globin from its combination with hæm. Ten times as much  $\text{NH}_3$  will not measurably displace the nitrogen compound of heliocorubin.

*Its stability in acid medium.* The tremendous affinity between nitrogen compound and hæm in heliocorubin is of biological importance, for without it heliocorubin could not exist as such. Globin-hæmochromogen in acid medium is almost entirely broken down into hæm and globin, because the affinity decreases with rising  $[\text{H}]$ . In heliocorubin, however, the affinity is so great that even in acid medium there is practically no free hæm. It is in the acid medium of the snail's gut that we find heliocorubin.

*Solubility.* Globin-hæmochromogen is much more soluble than any hæmochromogen we have been able to synthesise, but it is only slightly soluble at an acidity corresponding to that of the snail's gut. Heliocorubin is very soluble under these conditions.

*Its combination with  $\text{O}_2$  and  $\text{CO}$ .* Globin hæmochromogen forms a loose combination with  $\text{CO}$ , but it is well known that when brought in contact with the oxygen of the air it is oxidised to hæmatin. Hæmatin is the true oxide of hæmochromogen just as methæmoglobin is of hæmo-



globin. The oxygen of hæmatin cannot be removed by a gas pump, hæmatin can be reduced only by some strong reducing agent. The loose, easily dissociated, union between  $O_2$  and hæmoglobin characteristic of  $HbO_2$  is found in none of the hæmoglobin derivatives. In them the only oxy compounds known are those analogous to methæmoglobin rather than to  $HbO_2$ . It would seem, then, that to obtain loose combination with  $O_2$  it is not only necessary to add globin to hæm (as in hæmochromogen) but also to polymerise the resulting molecule four times so as to form hæmoglobin. The combination of helcorubin with  $O_2$  shows that even a hæmochromogen can combine loosely with  $O_2$ . If the intestinal fluid of the snail—a slightly acid solution of helcorubin—is exposed to the oxygen of the air, the helcorubin combines with oxygen to form the compound described by Dhéré and Vegezzi. If the fluid is now put into a tonometer which is then completely evacuated and rinsed several times with pure hydrogen, it will be found, when the helcorubin is examined spectroscopically, that the helcorubin is either completely or partly (depending upon the thoroughness of the evacuation) reduced. Helcorubin then in slightly acid medium—in its natural habitat—can combine loosely with oxygen. In a hæmochromogen with a suitable nitrogen compound, therefore, it is not necessary to polymerise the molecule to obtain a compound capable of combining loosely with  $O_2$ . To do this helcorubin does not have to be polymerised and, moreover, we were unable to polymerise it (though it is easy to polymerise globin-hæmochromogen). As previous investigators have shown, helcorubin when treated with ferriyanide or permanganate is oxidised to a hæmatin-like compound. Thus, like hæmoglobin, and unlike globin-hæmochromogen, helcorubin can be both oxygenated and oxidised.

*Effect of [H] on its affinity for  $O_2$  or CO* It has been shown that the positions of the absorption bands of respiratory pigments are connected with their affinities for gases(1) and that when a band is shifted by changes in [H] there may be concomitant changes in the affinity for a gas(3). The  $\alpha$  band of acid helcorubin is about 25 Å to the red of the  $\alpha$  band of globin hæmochromogen. If helcorubin is made alkaline the  $\alpha$  band shifts about 20 Å to the blue. At the same time the affinity of helcorubin for  $O_2$  or CO changes. If alkaline helcorubin is exposed to the air it remains reduced, or if acid oxyhelcorubin is made alkaline it is thereby immediately reduced. Apparently alkaline helcorubin has only a very small affinity for  $O_2$ . Dhéré and Vegezzi thought that this action of alkali is due mainly to its activating reducing substances present in the snail's intestinal fluid. This theory is made improbable

when it is found that CO combines with acid-helicorubin (when bubbled through the solution) but not with alkaline helicorubin. It can, moreover, be shown directly that there are no such reducing substances in the helicorubin solution. If the solution is made faintly alkaline with sodium bicarbonate, the helicorubin is reduced. If now a drop of oxy-hæmoglobin solution is added, the hæmoglobin remains oxygenated. Since hæmoglobin can remain oxygenated and helicorubin reduced in the same faintly alkaline solution, it is hardly possible that the helicorubin is reduced by any reducing substances. Keilin has recently shown that ethyl urethane can abolish tissue reductions. If ethyl urethane is added to an alkaline helicorubin solution even until a saturated solution of ethyl urethane is obtained, the helicorubin still remains reduced when exposed to the air. There are no reducing agents present. Apparently the affinity of helicorubin for  $O_2$  or CO is dependent on the  $[H]$ ; the more acid the medium, the greater the affinity. In the case of hæmoglobin the situation is reversed; the more acid the medium the less the affinity of hæmoglobin for  $O_2$  or CO. That the affinity of hæmoglobin for  $O_2$  is weakened by increased acidity is of the greatest biological importance, for it means that in the tissue capillaries  $CO_2$  tends to drive oxygen out of the blood. This property of the hæmoglobin molecule is not due to hæm but to the action upon hæm of the particular nitrogen compound—globin—with which it is combined. On the other hand, that helicorubin should be able to combine with  $O_2$  in acid medium seems to be biologically important because helicorubin exists in an acid medium and this property of helicorubin is due to the effect on hæm of a particular nitrogen compound.

It is interesting to note that the hæmochromogen formed by dissolving a few crystals of hæmin in pure pyridine is, in some ways, similar to helicorubin. Acid pyridine-hæmochromogen combines readily with either  $O_2$  or CO, whereas alkaline-pyridine-hæmochromogen does not combine with these gases (at ordinary tensions).

#### *The significance of helicorubin to the snail.*

Sorby regarded helicorubin from the evolutionary point of view. He thought that hæmoglobin might have been evolved from helicorubin "a bile pigment in a state totally unfit for the purpose of respiration, since it will not unite with loosely combined oxygen on exposure to the air." We have shown that helicorubin is as highly specialised a respiratory pigment as is hæmoglobin and that it seems to be as finely adapted to the environment of the snail's gut as is hæmoglobin to the blood of the

invertebrates Just as hæm has been radically modified by combination with globin and then polymerisation to form hæmoglobin, so also has hæm been radically modified—though in very different ways—by combination with a different nitrogen compound to form a truly remarkable hæmochromogen-helicorubin It exists in an acid environment, its solubility and stability in acid are of a different order from those of other hæmochromogens Hæmoglobin would be unstable in the environment of helicorubin, yet helicorubin, though only a hæmochromogen, can in acid medium form that loose combination with  $O_2$  peculiar to hæmoglobin The hæms of hæmoglobin and helicorubin are identical, but the different nitrogen compounds attached to the hæms of these two respiratory pigments have succeeded in adapting them to entirely different environments Helicorubin certainly cannot be regarded as a form from which hæmoglobin has evolved It is doubtful whether it is in any way analogous to the bile pigments of the vertebrates

The function of helicorubin in the snail is unknown, it is difficult to see how it can be considered either as a gas carrier (for it does not circulate in the snail) or as a respiratory catalyst (for it is present in large quantities) Yet it does seem as if helicorubin must have some function Its properties seem somehow to be delicate adaptations and there is too much of it to regard it as an accident It is not an excretion, for if a snail is fed on filter paper, the paper passes through the snail's gut and is excreted uncoloured The distribution of helicorubin in the molluscs<sup>1</sup> gives a slight indication of its function It is found only in the pulmonate molluscs and in all of these, except one—*Planorbis* *Planorbis* has hæmoglobin in its blood The other pulmonate molluscs have hæmoeyanin in their bloods It would seem, therefore, as if there might be some functional relation between hæmoeyanin and helicorubin Perhaps this depends on their having different affinities for  $O_2$  or, more particularly, on their affinities being affected differently by  $CO_2$

### SUMMARY

1 The similarities and differences between ordinary hæmochromogen and helicorubin have been explained by showing that they contain the same hæm but different nitrogen compounds

2 The nitrogen compound of helicorubin has a much greater affinity for hæm than globin has

<sup>1</sup> The helicorubin in the liver of the crayfish appears to be exactly the same as molluscan helicorubin

3. Helicorubin is both stable and soluble in an acid medium, in which ordinary hæmochromogen would be both unstable and insoluble.

4. Helicorubin combines loosely with  $O_2$ —a property which ordinary hæmochromogen possesses only when it is polymerised.

5. The affinity of helicorubin for  $O_2$  and CO increases with the acidity of its medium. This shows that the opposite effect of acidity on the affinity of hæmoglobin for  $O_2$  and CO is not due merely to the properties of hæm, but rather to the influence of globin on those properties.

6. The properties of helicorubin seem to be adaptations to the acid medium of the snail's gut in which it is found. The function of helicorubin, however, remains unknown.

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# THE EFFECT OF PITUITARY EXTRACT ON THE SECRETION AND COMPOSITION OF THE URINE.

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Montreal)*

THE experiments herein described are the result of an effort to obtain additional information concerning the action of pituitary extract on the secretion of urine. The data available so far appear on the surface to be of a conflicting nature. A number of observers (Magnus and Schafer(1), Schafer and Herring(2), King and Stoland(3), Knowlton and Silverman(4), and Hoskins and Means(5)) have observed diuresis to follow the administration of pituitary extract to anaesthetised animals, while others have observed a diminished rate of secretion. Among the latter, Fromherz(6) and Molitor and Pick(7) have obtained such results with unanaesthetised animals, and Frey and Kumpless(8) and Priestley(9) with human subjects. Further, the inhibitory action on urine secretion in diabetes insipidus has been demonstrated frequently in recent years. In addition to the effect of pituitary extract on the rate of water secretion, some of the other constituents of the urine have been studied, particularly the chloride content. The results obtained by others in this regard can be commented upon most profitably in discussing our own data.

We have employed for the most part normal, fasting (20 hours after food), unanaesthetised dogs with bladder fistulas, and compared the urine with regard to quantity and composition before and after the intravenous administration of pituitary extract. Permanent fistulas were established in a number of female animals by bringing the bladder out to the surface, making a longitudinal incision, and then sewing the cut edges to the skin and underlying muscles. The animals were ready for use in about two weeks and no difficulty was experienced in collecting the urine as formed. Chlorides were determined by the Volhard-Arnold method, urea by the method described by one of us(10), and total phosphorus by oxidation of the urine with hydrogen peroxide and sulphuric acid and precipitation of the phosphoric acid with ammonium molybdate, the yellow precipitate being weighed as well as precipitated by Pregl's(11)

technique. All volumes are expressed in mils. per minute and chloride, chlorine, urea and total phosphorus in milligrams per minute. The pituitary extract employed was Parke, Davis and Co.'s obstetrical "pituitrin." The preliminary treatment of the animals was varied; in some cases the extract was administered shortly after they had received water by mouth, while in others several hours were allowed to elapse before it was given, practically all of the water having been excreted. The object of the second procedure was to insure that the animals were not dehydrated.

Under these conditions the results are as follows. If the urine secretion is proceeding slowly, the administration of pituitary extract causes first, as a usual thing, complete anuria for varying lengths of time—a few minutes up to 15 or 20 minutes—and then a period of diuresis followed by a return to normal. The volume rate is influenced to variable degrees. In some instances the effect is more marked than in others, but an increase is nearly always present. Along with the increase in volume rate there is an extremely striking effect on the chloride excretion; this is out of all proportion to the change in volume rate and has amounted to as much as a seventy-four-fold increase in the minute rate compared with the control chloride rate, and a hundred and sixty-fold increase in the chloride percentage. The chloride concentration tends to reach a maximum at about 0.7–1.0 p.c. chlorine or 1.15–1.65 p.c. NaCl. Urea and phosphate excretion, in the experiments in which these substances have been determined, are also increased in unmistakable fashion, but the effect is much less marked quantitatively than in the case of chlorides. The action on the volume rate may be merely a secondary one, the primary effect of pituitary extract being upon the chloride excretion; the increased urine flow would then be regarded as representing solvent for the chlorides. For a reason to be mentioned later, the diuresis may also be regarded as due to direct stimulation of water secretion. It is perhaps worth recording that the first portion of urine secreted after the period of anuria contains protein. This may indicate some transient asphyxial damage but it is not likely that the later effect on chloride excretion is a consequence of this, since Marshall and Crane<sup>(12)</sup> have shown that asphyxia for 20 minutes is accompanied by only a slight increase in the chloride eliminated subsequently or no increase at all. Tables I and II are representative of seven experiments in all of which the results were of the same general nature.

Since the diuresis which various investigators, already mentioned, have observed to follow the administration of pituitary extract occurred

in anæsthetised animals, we desired to know whether the chloride excretion likewise was affected under anæsthesia. Table III represents one of several similar experiments. It may be seen that there is a slight but definite diuresis when comparison is made with the rate following the induction of anæsthesia but compared with the normal control rate the effect is small. The rate of chloride excretion, on the other hand, is increased but slightly when pituitary extract is administered to the anæsthetised animal. These results we attribute to suppression of the capacity of the kidney to respond to that which in the normal animal is an adequate stimulus to activity.

If the urine secretion is proceeding rapidly, the increase in the volume rate is absent. In fact, in this group of experiments, the volume rate after pituitary extract has never reached the control rate. The initial anuria or oliguria is present, as when urine secretion is proceeding slowly (in all cases there is decided slowing for a time), and then the rate increases considerably but does not reach the initial value. So far as the chloride excretion is concerned, however, there is no difference in this respect between the results of these experiments and those in which the initial volume rate was low in the control period. Both chloride percentage and rate are extraordinarily increased, the former tending to a maximum in these experiments also at about 0.7-0.9 p.c. Cl. Table IV is a fair sample of the results obtained in four experiments. It appears from these two sets of experiments in which the pituitary action is superimposed upon low and high rates of volume secretion respectively, that whether one observes diuresis or antidiuresis depends upon the rate of secretion at the time—when it is high the antidiuretic action predominates, but when it is low diuresis ensues. The effect on the chloride excretion is under ordinary conditions independent of the effect on the excretion of water, both quantity and percentage of chloride being increased.

We were surprised by the fact that the chloride concentration appeared to reach a maximum considerably under that which may be attained by the administration of sodium chloride by mouth or by vein and consequently investigated the effect of pituitary extract on the composition of the urine when the chloride excretion was raised by the previous administration of sodium chloride. When this is done, the chloride percentage of the urine does not increase but actually decreases. However, we cannot say with confidence that it attains the same level reached in the experiments first described, though we believe there is a tendency in this direction. Since the pituitary action is comparatively short it is possible that we have missed the minimum point of chloride

percentage by not analysing samples taken with sufficient frequency. Table V is illustrative of six experiments. In Fromherz's communication the effect on chloride secretion is much the same as that observed in the present experiments; the maximal chloride excretion being practically the same; the decrease in chloride concentration following pituitary, when the animal was on a salt-rich diet, was also obtained by Fromherz, but no comment was made upon it.

The experiments thus far described appear to us to justify the following hypothesis concerning the action of the posterior lobe of the pituitary gland on the secretion of urine. The effects of the active constituent on the secretion of water and chloride are similar. When either or both are being secreted slowly, pituitary extract causes an increase in the rate or rates; when either or both are being excreted rapidly it has the opposite effect. Consequently it may be assumed that the pituitary gland has a definite function in determining the secretion or conservation of water and chlorides, and it may well be that this control is exerted over other constituents of the urine as well. Starling and Verney<sup>(13)</sup> have also suggested recently, on rather slender evidence, that pituitrin, or some similar substance, may have a regulating influence on the output of chlorides and water by the kidney. In this connection one experiment (protocol not included), similar to those illustrated by Table V, in which the percentage of chloride was diminished, while the phosphorus excretion rose decidedly, should be mentioned. Apparently chloride and phosphate excretion may be affected in opposite directions by pituitary extract, which is in harmony with the idea of its exerting a general regulatory action. In other experiments (six in number represented by Tables I and II) the phosphorus excretion was increased decidedly along with the chlorides and in several (Tables I, II and VII, and other experiments not herein published) there can be no doubt that the excretion of urea was increased. These facts certainly point in the direction of pituitary extract having a general stimulatory effect on secretion under the conditions existing in these experiments.

In such experiments as we have described on intact animals one cannot draw definite conclusions regarding the mechanism of the events. On the basis of the theory of urine secretion formulated by Cushny<sup>(14)</sup>, the increased rate of chloride excretion might be ascribed to diminished tubular absorption of chlorides accompanied, possibly, by increased blood-pressure and blood flow through the kidney. That the last two factors are not necessarily involved is indicated by the paper of Starling and Verney above referred to. They show that the increased chloride



rate, as well as chloride percentage, in the urine secreted after pituitary extract by their heart-lung-kidney preparation occurs in the absence of changes in blood-pressure and blood flow. The diuresis in our own experiments could be explained, on the basis of Cnshny's theory, as an osmotic effect following from the high salt content of the tubular fluid. However, this theory offers no explanation of the decreased chloride percentage of the urine which occurs under suitable conditions, or of the antidiuretic effect, though the latter might possibly be explained on the basis of increased re-absorption of water. Then, in order to explain the diuretic action, it would be necessary to regard this as a salt effect. We prefer, however, to regard the diuresis as due to a definite stimulatory action on the secretion of water by the kidney, and the antidiuresis as due to an inhibitory action. This is in line with the action on chloride excretion which may be augmented or diminished, the result depending upon the chloride concentration of the urine being secreted prior to the administration of the extract. Such a conception is equivalent to assigning to the pituitary gland an action which, for want of a more elucidatory expression, may be called regulatory.

Changes caused by pituitary extract on the balance of materials in the tissues may play a rôle in determining the results, but the experiments of Starling and Verney indicate that the locus of action is in the kidney itself. Changes in the water and chlorine content of the blood do not appear to be involved. In three experiments in which plasma Cl and blood solids were determined, no significant changes occurred as a result of the pituitary extract. Solids decrease slightly but no importance can be attached to this.

Several reasons point against the possibility of explaining our results on chloride excretion as due to a 'washing out' process. The fact that the increase in chloride excretion occurs even when the volume rate of secretion diminishes, and the marked increases in chloride concentration are two potent ones. Nevertheless we have investigated the effect of very pronounced water diuresis on the chloride excretion and found no important increase in the minute rate, and, indeed, a decided drop in its percentage, apparently the chloride absorbing capacity of the tubules is not affected by greatly increasing the volume rate of secretion (Table IV). When the volume rate is increased by the intravenous injection of Ringer's solution in which the sodium chloride is replaced by sulphate (osmotically equivalent, Table VI), the chloride excretion is likewise affected only slightly. Volume rates equal to those which develop when water is given by mouth do not occur in this case, but it

would seem that the re-absorption of chloride (assuming the validity of Cushny's theory for the moment) is carried on quite efficiently during the diuretic period.

Isotonic urea injected intravenously (250 c.c.) (Table VII) resulted in a fair degree of diuresis, but the chloride rate and percentage were affected no more than by the sodium sulphate-Ringer solution. The hæmolytic action of urea was very pronounced in the experiment cited, but while the urine contained much hæmoglobin, it contained no sugar. Since it is not likely that hæmoglobin is filterable through the glomerular membrane it is probably to be regarded as a product of secretory activity.

Isotonic dextrose in one experiment (Table VIII) caused an astonishing rate of chloride excretion. The volume rate in the experiment cited was only slightly greater than that when diuresis was caused by giving water by mouth, so that the explanation for the difference between the chloride rates is not at all apparent. Possibly it was due to some temporary deleterious effect of the dextrose on the kidney which allowed sodium chloride to pass. This is somewhat plausible when the low chloride percentage is observed. The concentration was immediately increased by the administration of pituitary extract though the actual minute rate of chloride excretion was not increased. In a similar experiment dextrose did not affect the chloride excretion to any great extent, but pituitary extract administered at a time when all of the dextrose solution had been accounted for produced the usual result.

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## APPENDIX

TABLE I

200 c c H<sub>2</sub>O at 10 a m

Time	Vol per min	Cl per min	Cl p c	Urea per min	Urea p c	P per min
1 39-1 54	21	27	13	7 8	3 71	074
1 54-2 00	19	23	12	8 1	4 28	016
2 12-1 c c Pituitrin intravenously						
2 12-2 24	0 0	—	—	—	—	—
To* 2 12	14	37	26	5 3	3 78	139
„ 2 52	68	4 3	63	17 2	2 53	264
„ 3 02	47	3 94	84	8 0	1 70	057
„ 3 22	18	1 35	75	7 3	4 05	014
„ 3 37	18	39	22	11 1	6 18	022
3 40-1 c c Pituitrin intravenously						
3 50-4 03	24	96	40	12 0	5 05	13
To 4 15	81	5 73	71	17 7	2 17	31
„ 4 25	65	4 77	73	11 7	1 80	16

\* *I e* from 2 24-2 42, similarly in other cases

TABLE II

20 8 kg dog 300 c c H<sub>2</sub>O at 10 a m

1 23-1 43	178	204	12	9 6	5 42	235
1 43-2 03	220	206	09	12 1	5 48	057
2 05-2 c c Pituitrin intravenously						
2 08-2 22	13	29	21	4 8	3 68	181
To 2 32	65	4 3	66	20 5	3 14	1 43
„ 2 42	1 58	14 9	94	23 6	1 48	1 19
„ 2 52	78	8 16	1 09	9 27	1 19	52
„ 3 02	—	178	—	7 48	—	31

TABLE III

8 1 kg dog 200 c c H<sub>2</sub>O at 11 30 a m

Time	Vol per min	Cl per min	Cl p c
2 36-3 11	34	25	073
3 11-3 39	28	28	100
Lithers anaesthesia begun.			
3 44-4 13	15	25	16
4 13-1 c c Pituitrin intravenously			
4 13-4 34	17	45	27
To 4 47	40	47	14
„ 5 13	057	—	—

TABLE IV

8 11 g dog 150 c c H<sub>2</sub>O at 11 a m and 150 c c at 1 p m.

1 57-2 12	1 6	—	—
2 14-250 c c H <sub>2</sub> O			
2 15-2 30	2 77	49	018
2 30-2 40	4 85	44	009
2 42-1 c c Pituitrin "			
2 46-2 56	53	2 42	16
To 3 16	79	4 17	53
„ 3 41	97	7 00	71
„ 4 13	72	2 41	33
„ 4 23	60	60	10

TABLE V.

20.8 kg. dog. 400 c.c. 2 p.c. NaCl at 9.15 and 11.15 a.m.

Time	Vol. per min.	Cl per min.	Cl p.c.
2.40-3.00	.83	11.73	1.41
3.00-3 10	.83	11.3	1.35

3.25-1 c.c. "Pituitrin" intravenously.

3.30-3.50	.51	5.75	1.13
To 4.00	1.53	15.4	1.00
" 4.10	1.75	18.9	1.08
" 4.20	1.54	16.1	1.05
" 4.40	.98	12.6	1.29
" 4.50	.86	11.3	1.31

Plasma Cl

Blood solids

3.22	3.50	3.22	3.50
.408	.408	19.7 p.c.	18.7 p.c.

TABLE VI.

7.6 kg. dog. 150 c.c. H<sub>2</sub>O by mouth at 12.45 p.m.

3.30-4.00	.16	.38	.23
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100 c.c. Na<sub>2</sub>SO<sub>4</sub> Ringer intravenously.

4.37-4.47	.65	.67	.10
4.47-5.07	.26	.38	.14

5.10-1 c.c. "Pituitrin" intravenously.

5.12-5.22	1.47	9.20	.63
5.22-5.32	.46	2.67	.58

TABLE VII.

10.5 kg. dog. 200 c.c. H<sub>2</sub>O at 10 a.m.

Time	Vol. per min.	Cl per min.	Cl p.c.	Urea per min.	Urea p.c.
1.50-2.10	.23	.26	.11	8.27	3.68

2.14-0.25 c.c. "Pituitrin" intravenously.

2.15-2.25	.00	.00	.00	—	—
To 2.40	.13	—	—	—	—
" 2.55	.28	1.30	.46	13.1	4.68
" 3.05	.36	2.01	.57	10.0	2.78
" 3.20	.38	1.88	.50	—	—
" 3.35	.48	1.70	.36	11.4	2.37
" 3.59	.39	.73	.19	—	—

4.04-4.10-250 c.c. 1.8 p.c. urea intravenously.

3.59-4.26	.78	.50	.064	—	—
To 4.51	.88	.25	.028	22.7	2.58
" 5.06	1.25	.57	.046	—	—
" 5.21	1.51	1.34	.089	21.2	1.4
" 5.31	1.75	—	—	—	—
" 5.46	1.23	.86	.07	—	—

TABLE VIII.

8.1 kg. dog. 150 c.c. water at 10.30 a.m.

1.59-2.31	.17	1.24	.72
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2.31-2.41-250 c.c. 5.4 p.c. dextrose intravenously.

2.31-2.41	2.55	6.9	.27
To 2.46	5.7	15.2	.27
" 2.56	1.97	3.08	.16

2.57-0.5 c.c. "Pituitrin" intravenously.

2.56-3.06	.92	4.95	.54
To 3.26	.76	7.4	.97
" 3.36	.73	6.2	.85
" 3.46	.54	3.5	.65

# LENGTH OF MUSCLE, AND THE HEAT AND TENSION DEVELOPED IN AN ISOMETRIC CONTRACTION.

By A. V. HILL.

(From the Department of Physiology, University College, London.)

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## I. THE RELATION BETWEEN LENGTH AND HEAT PRODUCTION.

AN account of the older work upon this subject is given in O. Frank's review (1), pp. 440, etc.). Heidenhain was the first to show that the extent of the heat production in a muscle is not determined once and for all by the stimulus, but depends also upon the mechanical conditions obtaining during contraction. This general statement of the matter has been fully substantiated since, especially by the work of Fenn(2). The simplest expression of Heidenhain's results is that, in a set of isometric contractions carried out at a series of different lengths, the heat production increases at first with increasing length, attains a maximum, and then decreases again as the length is still further increased. This conclusion was disputed by Blix, who believed that he had established the fact that the heat production goes on increasing indefinitely as the length in an isometric contraction is increased. Subsequent investigation, however, has shown that Heidenhain's conclusion was correct.

The matter was taken up again in 1914 by Evans and A. V. Hill(3). Their experiments made on frogs' sartorius muscles showed that, starting from a length not far from the resting unloaded length, an extension causes at first a rise in the heat production as well as in the force developed, while a further extension causes a fall in both. They found that the ratio of tension developed to heat produced is constant only for a certain range of extension of the muscle, the maximal value of  $T/H$  being reached at about the natural length of the muscle *in situ*.

A further investigation was undertaken by Doi<sup>(4)</sup>, who showed, both in skeletal and in cardiac muscle, that the isometric mechanical response increases with extension up to a certain limit and then decreases as the extension is continued further. In his second paper Doi confirmed the statement that heat production in skeletal muscle reaches a maximum at a certain moderate extension, falling rather rapidly on both sides of this particular optimal length. Much recent work, especially that of Fenn<sup>(2)</sup>, Azuma<sup>(5)</sup>, Hartree (unpublished), etc., has incidentally confirmed this general statement of the variation, in an isometric contraction, of heat production with length.

Before the adoption of the shielded muscle chamber, which can be immersed in well stirred water in a constant temperature vessel, it was unsafe to allow muscles to shorten to any appreciable degree over the junctions of a thermopile, for fear of incurring errors due to bringing warmer or colder areas of the muscle upon those junctions. For this reason the experiments of Evans and Hill were made only at and beyond the resting length of the muscle. To investigate the heat production at shorter lengths, it is necessary to allow the muscle to shorten a certain distance over the thermopile on which it is stimulated, before holding it isometrically at the shorter length. With the muscle chambers now in use it is possible to secure such constancy of temperature within the chamber that no errors of this type are likely to arise; the relation, therefore, between heat production and length can now be investigated over the whole range of lengths, from the shortest to which the muscle will contract unloaded, on subjecting it to a tetanic stimulus, to the greatest to which it is safe to stretch it.

*Methods.* The myothermic chambers used were of the type described by Fenn. Several actual instruments have been employed, for all of which I am indebted to Mr A. C. Downing of this Department. They have been modified in various ways from Fenn's original design and two of them are shown in Figs. 1 and 2. Usually fixed electrodes have been employed, as shown in the figures; occasionally, however, the distal electrode has been brought into contact with the muscle just above its tendon, by means of a platinum loop soldered to a steel wire leading to the tension measuring apparatus. Some of the experiments have been performed in oxygen, in cases where it was necessary to obtain a measurement of the heat in absolute units: a calibration of the muscle is not possible in Ringer's solution. Several, however, of the relations established below do not demand an absolute calibration of the apparatus, and since the muscle lives longer and behaves better in

Ringer's solution, the experiments on frog's muscles have, where possible, been carried out in that medium.

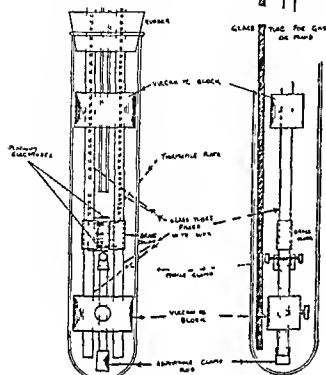
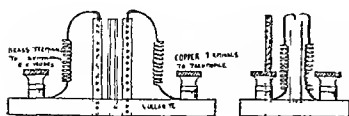


FIG 1

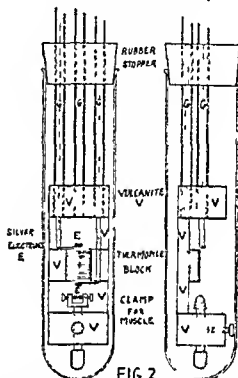
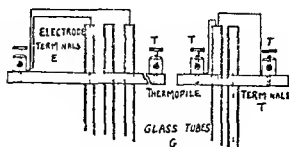


FIG 2

Fig 1. Thermopile and muscle chamber, for pair of sartorius muscles. Front and side elevations. Thermopile made by silver plating constantan wire wound spirally on a thin strip of ivory previously soaked in hot paraffin. Outer ("cold") junctions kept cool by being covered with brass collars. *NB* A sufficient distance must be allowed between the vulcanite plate at the top and the rubber stopper, to ensure that the chamber is fairly deeply immersed in well stirred water.

Fig 2. Thermopile and muscle chamber for single muscle, e.g. biceps cruris of tortoise. Front and side elevations. Thermopile wound on a thick block of vulcanite, the "cold" junctions being at the back sunk in a groove in the vulcanite carrier *V*.

Two types of preparation have been employed: firstly, the usual double sartorius of the frog, and secondly, a single long straight leg muscle of the tortoise, the *biceps cruris* (see Bojauus (6)). The latter is usually about 50 mm. in length: it is thicker than the sartorius, and the single muscle employed (not a pair as with the frog's sartorius) usually weighs some 100 mgms. This muscle, the use of which will be further described elsewhere, contracts extremely slowly when compared with the frog's sartorius at the same temperature, and it would seem important that

precisely similar results, qualitatively and quantitatively, have been obtained with two such different muscles.

When a calibration in absolute units of heat was required, this was carried out by a new method involving a "vacuo-junction," a delicate yet simple recorder of small alternating currents, supplied by the Cambridge Instrument Co.; the use of this will be described elsewhere. The method is simple and convenient to use, and gives results of considerable accuracy.

For stimulation a tetanus from a Porter coil was always used, its duration being adjusted by a Lucas revolving contact-breaker. For the calibration an alternator was employed, giving a single phase alternating current of about 100 cycles per second. A relatively high frequency is desirable for the calibration, to avoid polarisation at the electrodes. Such an alternating current, however, is not so suitable for stimulation, especially of a slow moving muscle such as that of the tortoise, where results analogous to a Wedensky inhibition appear to occur. Moreover, with a smooth alternating current of this type, if employed for stimulation, very large E.M.F.'s have to be used to secure a maximal response, and these are apt of themselves to liberate considerable quantities of heat in the live muscle. This is avoided by the use of the tetanising coil.

The muscle was connected to a set of levers as shown in Fig. 3.

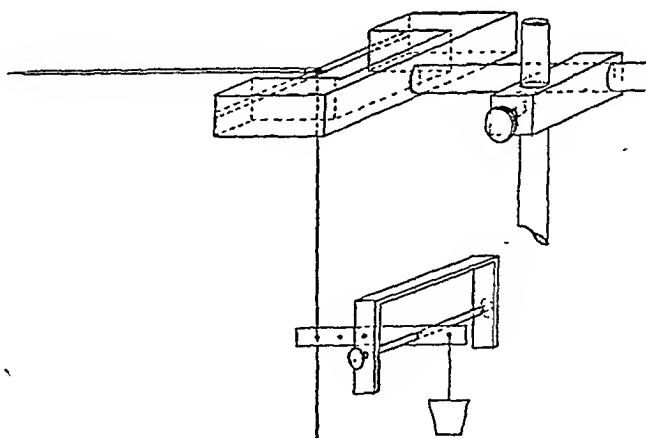


Fig. 3. Lever system, combined isometric and isotonic, with thread connecting. See text.

A strong piece of linen thread, or a steel wire, passed upwards from the muscle to an isotonic lever, loaded near its axis with a weight which put



a tension of about 2 gms. constantly on the muscle. The resting unloaded length of the muscle—which is always somewhat arbitrary—is taken to be that corresponding to this small load. Vertically above the isotonic lever is an isometric one connected to it by a loose thread: this lever is of fairly short period and allows extremely little shortening. Both levers are mounted together upon the sliding portion of a Palmer stand, which can be adjusted vertically by a screw. A millimetre scale is provided to measure the amount of vertical movement. The resting unloaded length corresponds to a position of the stand in which the thread joining the two levers is just tight, so that the muscle comes up against the isometric lever directly it begins to shorten. Lengths greater than the unloaded one are obtained by screwing upwards by the required amount the stand which carries the two levers. The muscle is then stretched by the isometric lever by that amount. Lengths less than the resting one are similarly obtained by screwing the stand downwards through the required distance, so that the muscle can shorten freely under the small load of the isotonic lever, until the string joining the latter to the isometric lever becomes tight; then the rest of the contraction is carried out isometrically at the length determined by the position of the stand.

The experiments have usually been conducted at temperatures in the neighbourhood of  $15^{\circ}\text{C}$ ., but they have been made also, in the case of frog's muscle, at temperatures as low as  $0^{\circ}\text{C}$ ., and in the case of tortoise's muscle at temperatures as high as  $25^{\circ}\text{C}$ . Similar results, qualitatively and quantitatively, are obtained at all temperatures.

The tortoise muscle usually lasts so well and remains in such constant condition that it is unnecessary to make a "reverse" series. With a frog's muscle, in order to obtain results unaffected by on-coming fatigue, it is often desirable to take a reverse series, and to employ the average of the two readings made at any one length.

Apart from observations made at greater than the resting unloaded length, the maximum deflection of the galvanometer is always attained at a moment when the muscle has already relaxed into its original "unloaded" position. Thus over the lower range the heat may always be taken as being directly proportional to the number of scale divisions of galvanometer deflection. It is unnecessary then to make a new calibration for every position of the muscle during its contraction. When, however, the muscle is extended beyond its resting unloaded length, the conditions are different. In this case the whole contraction and relaxation occur, and the heat production is measured, at a length

greater than the unloaded one. Consequently a smaller portion of muscle lies upon the junctions of the thermopile and the calibration number (i.e. the value of one scale division in ergs) is different. The variation of the calibration number with length depends upon the shape and thickness of the muscle, and upon the particular thermopile used. Theoretically, therefore, in order to measure the total amount of heat liberated in the whole muscle, it is necessary to make a calibration at every length considered. This would be laborious in practice and actually not essential. The method adopted has been to carry out a calibration on the dead muscle at two lengths, one as short as possible, approximating to the resting unloaded length, and the other at the greatest length at which observation has been made. A linear interpolation between these two gives, with sufficient accuracy, the calibration number for any intermediate length required.

This variation of the calibration number with length is an important factor in the technique, and it was never attended to in the older observations relating the heat production to length. It may have introduced serious and incalculable errors into the results both of Heidenhain and Blix. Its effect, with the thermopiles used by myself, is generally to make the apparent falling off of the heat production, with extension beyond the resting unloaded length, greater than the actual falling off. The explanation of the variation of calibration number with length is simple. With a sufficiently thin muscle the galvanometer deflection is approximately proportional to the total amount of heat liberated in the piece of muscle lying on the thermopile. If the muscle be extended the quantity of it actually lying on the thermopile is less: consequently the total heat liberated in the whole muscle, for a given amount of heat liberated in the portion lying on the thermopile, is greater. Since we are concerned with the heat liberated in the whole muscle, and not merely in the portion of it which happens to lie on the thermopile, this implies a greater calibration number for the whole muscle when it is in the extended condition. An example of the method of observation and calculation employed may make the argument clearer.

Exp. on pair of sartorii of Dutch *Rana esculenta*: weight, 0.228 grm.; length resting unloaded, 3.8 cm.; 0.5 sec. tetanus with Porter coil at 11 cm. employing 2 volt accumulator; with an extra 100 ohms in the galvanometer-thermopile circuit, as employed during observations (to reduce sensitivity)  $4 \times 10^{-6}$  volts gave 98 mm. deflection. Calibrated afterwards at lengths 40, 46 and 50.5 mm. Distance between (fixed) electrodes 23.0 mm. In the portion of muscle between the electrodes the value of 1 mm. deflection was observed to be 291 ergs, 286 ergs and 286 ergs respectively; so that in the whole muscle the value

of 1 mm deflection was  $291 \times \frac{40}{23} = 505$  ergs,  $286 \times \frac{46}{23} = 572$  ergs,  $286 \times \frac{50.5}{23} = 628$  ergs, respectively. Plotted against the corresponding lengths these calibration numbers follow a sensibly linear relation which allows values at intermediate lengths to be interpolated. The following observations were made on the live muscle

Length, mm	23	28	33	38	43	48	50.5
Per cent	60	74	87	100	113	126	133
Heat, mm	292	598	796	805	620	494	469
Calibration no., ergs	485	485	485	485	538	597	628
Heat $H$ , $10^3$ ergs	142	290	385	390	333	295	294
Initial tension, $10^3$ dynes	0	0	0	9	44	105	139
Tension developed $T$ , $10^3$ dynes	13	76	128	149	109	61	46
$T_0/H$	34	1.00	1.27	1.45	1.24	.78	.595

Notes (a) Here  $l_0$  is the resting unloaded length, 3.8 cm

(b) The calibration number for the first four lengths is the same, since for each the muscle relaxed to 3.8 cm before the heat was measured

This exp. is shown graphically as full circles in Fig. 8

It will be seen from this experiment that a considerable error may be introduced by neglecting the variation of calibration number with length. Unless, therefore, proof be adduced to the contrary, it is always advisable to multiply the deflection of the galvanometer actually observed by a suitable calibration number; this must be determined at the length at which the muscle lay on the thermopile at the moment at which the maximum deflection of the galvanometer recording the heat production was measured. In none of the older investigations was this done, and it is impossible to say now how far the error so introduced may have affected the results. Moreover, since a calibration is impossible in the case of a muscle of non-uniform section, observations on such muscles are strictly comparable only if the muscle lie always in the same position on the thermopile, at the moment when the heat is recorded by the maximum deflection of the galvanometer.

In the example given above the calibration was carried out at three lengths, and the results show the relation between calibration number and length to be sensibly linear over the range considered. In other experiments this fact was assumed and the calibration number actually observed only at two lengths, the two points so obtained being plotted and joined by a straight line to allow interpolation. In this example the calibration was carried out by fixed electrodes. If the electrodes had not been fixed, but the distal one connected to the muscle near its tendon, the calibration number could have been read directly instead of being obtained by calculation from the length of the muscle. This procedure was adopted in some cases. The method described in the example makes it more obvious, however, why the calibration number

varies with length, since it shows that for the piece of muscle lying between the electrodes the number is approximately constant, and therefore approximately proportional, in the case of the whole muscle, to its length.

In many of the experiments on frog's muscles, *e.g.* those shown in Fig. 4, the readings of heat production at lengths less than 100 p.c. were obtained simply in terms of scale divisions of galvanometer deflection. Since the sensitivity of the galvanometer may vary from time to time, this was tested at intervals throughout each experiment and the results all reduced to a constant sensitivity of the galvanometer.

In all the earlier experiments performed, which covered only the shorter range of lengths, observations were usually made in order of diminishing length, in or about the following positions: 110, 100, 95, 90, 80, 70, 60, 50. As shown in the figures, the maximum heat production occurs usually in the neighbourhood of relative length 95, so that an observation was interpolated between 100 and 90. In many experiments, including all those made upon tortoise's muscle, the observations were made not precisely at these stated lengths, but at certain neighbouring lengths chosen for convenience. In such cases, in order to obtain a curve representing the mean of several experiments, the observations of heat production were plotted against the relative lengths and a curve drawn through the plotted points. From the curve the heat production at certain standard relative lengths was read off, and reduced to a percentage of the maximum heat production. Since, in the first instance we are concerned only with the variation of heat production with length, these percentage values are all that is required for our immediate purpose. The stimuli were always maximal, and when a reverse series also was made, each point plotted represented the mean of the two observations at the length considered. Since chance variations occur from observation to observation and from muscle to muscle, it was desirable to make a mean curve from a number of observations on different muscles. A table therefore was constructed of the percentage heat production at each of the standard relative lengths, for a number of experiments on different muscles. The mean for each length was then calculated and plotted, and the mean curves resulting are those shown in Figs. 4, 5 and 6. The greater the care taken and the better the condition of the muscle, the more regularly is the same result obtained: qualitatively the results are always similar, quantitatively there are usually only slight differences from the means shown in the figures. The curves, therefore, represent with considerable accuracy the relation

between heat production and length in a normal isolated muscle in good condition and unfatigued. To avoid fatigue an interval of  $3\frac{1}{2}$  to 5 minutes was allowed between successive observations.

### Results.

The results can best be described with the aid of the figures. In Fig. 4 each full line represents the heat production from length 100

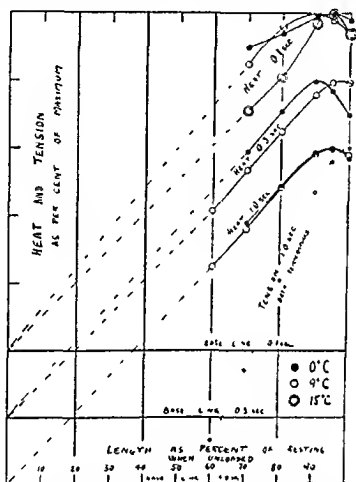


Fig. 4. Relation between heat production and length, at lengths up to that of the resting unloaded muscle; three durations of stimulus, three temperatures; mean results of a number of experiments. Sartorius muscles of *Rana temp.* [N.B. Three base lines.]

down to the smallest length to which the muscle will shorten. The experiments shown in this figure were performed in Ringer's solution, without a "reverse" and without calibration, at three temperatures and for three durations of stimulus. The observations at the highest temperature were made only for the shortest duration, since the muscle more readily fatigues at a high temperature. The experiments are shown plotted to three different base lines, in order to avoid confusion between the different curves. The broken lines to the left join the observed curves to the origin. The actual experimental observations were plotted,

and interpolations carried out for lengths 100, 95, 90, 80, 70, 60, as described above. These interpolated quantities were then reduced to a common maximum of 100, and their average value for each length in a number of experiments is shown in the figure. A similar curve is shown for the tension exerted in a 1.0 sec. contraction at 0° and 9° C. This figure shows that, on the short side of the unloaded length, the same type of relation occurs at all temperatures and for all durations of stimulus.

In the shortest tetanic contraction, approximating to a single twitch, it is impossible to obtain results over so wide a range of lengths as it is with a more prolonged contraction; the muscle will not shorten so far in the less prolonged contraction. A certain characteristic and constantly recurring difference exists between the result of the very short stimulus, 0.1 sec., and that of the longer. Qualitatively the same relation is obtained, namely, a curve with a maximum heat production in the neighbourhood of 90 or 95 p.c. of the resting unloaded length. The curvature, however, is often not so pronounced as in the case of the longer stimulation. In all cases, but especially in that of the frog's muscle at a low temperature, or in that of the tortoise's muscle which contracts very slowly, a stimulus of 0.1 sec. is over, or practically over, before the shortening has been completed, so that the stimulus has been applied mainly at lengths greater than the final length at which the contraction is completed isometrically. Moreover, as Fenn(2) has shown, when a muscle shortens doing work it gives out more heat, corresponding to the work done. The only external load in these experiments was that of the isotonic lever, exerting a constant tension on the muscle of only 1 or 2 gms. The external work, therefore, is practically negligible. An active muscle, however, possesses, as Gasser and Hill have shown(7), a considerable degree of "viscosity," and in a rapid unloaded shortening an appreciable amount of mechanical work must be done by the muscle against its own internal viscous resistance. This work will entail—pursuing the argument of Fenn—a certain amount of extra heat, which in the contractions evoked by shorter stimuli must be relatively more important than in those evoked by longer ones. Hence, we might expect that in the contractions with shorter stimuli the heat production would not diminish with decreasing length relatively as much as in the contractions evoked by longer stimuli. The curvature of the relation would be less, as has actually been found. To study the relation between heat and length in its purest form it is advisable to consider chiefly the results of the longer stimulation.

Before discussing more fully the results shown in Fig. 4 we must consider those exhibited in Figs. 5 and 6. In Fig. 5 are shown three

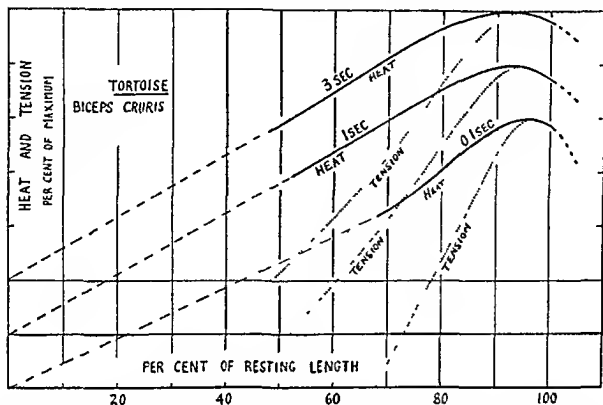


Fig. 5. Relation between heat production and length, at various lengths up to that of the resting unloaded muscle. Corresponding tensions also shown. Plotted to three different base lines to prevent overlapping. Mean curves from a number of experiments. Biceps cruris of tortoise. The dotted continuations to the right were observed but they may involve a slight error since no calibration was carried out for lengths greater than 100 p e

curves obtained by the methods of observation and calculation described above, on the biceps cruris muscle of the tortoise. Here again the observations were made, without reverse and without calibration, at lengths shorter than the resting unloaded length. Actually observations were continued to length 105, and the continuations of the curves are given. These continuations are shown in broken lines as, owing to the absence of a calibration, the observations beyond length 100 are not entirely reliable. In this figure also, to prevent confusion, the results for the three different durations of stimulus are shown plotted to three different base lines. The tension curves are presented in a similar way. It will be noted that the one second and the three seconds curves are practically identical, the former ending a little sooner than the latter, while the 0.1 sec. curve is appreciably different in form, and ends considerably sooner. The divergence of the 0.1 sec. curve is due presumably to the factors discussed above. Observations made at various temperatures are included in the means shown in Fig. 5: no difference was

observed between the results obtained at different temperatures. The curves shown in Fig. 5 continue to a rather shorter length than those of Fig. 4 on frogs; the tortoise's muscle appears to be able to shorten relatively rather further than the frog's. The broken lines to the left join the origin to the commencements of the several curves. Here, again, we may say that the characteristic relation between heat and length, at lengths less than 100, is most clearly shown in the case of the longer stimuli. The tortoise's muscles of Fig. 5 were in air in the muscle chamber.

Fig. 6 is of a composite nature. The observations at lengths less

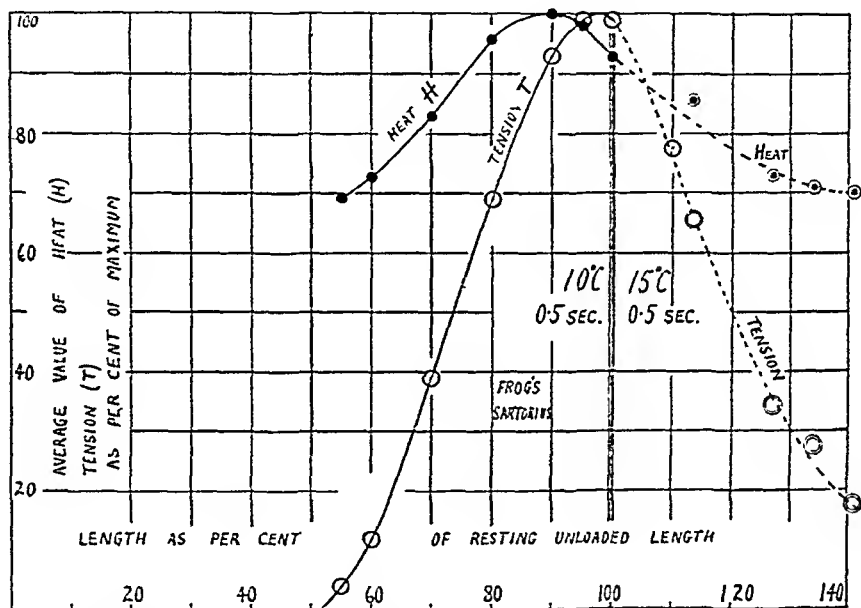


Fig. 6. Relation between heat and length, and tension and length, for frog's sartorius. All 0.5 sec. tetanus. Lengths less than 100 p.c., *Rana temp.* at 10° C. Lengths greater than 100 p.c., *Rana esc.* at 15° C. Curves made to join by adjusting the scale at 100 p.c. length. For details see text.

than 100 were all made on English *Rana temporaria*, in Ringer's solution with reverse carefully carried through, in order if possible to eliminate the effects of on-coming fatigue. The observations of heat and tension were made actually at the relative lengths shown, and were not obtained by interpolation from other observations. In each experiment they were reduced to a maximum of 100, and the mean of the several experiments computed for each length. These mean values are shown in the figure. All experiments were carried out in the immediate neighbourhood of



10° C., and all with a stimulus lasting for 0.5 sec. The observations in Figs. 4 and 5 having established the general relations, it was thought desirable to record them in a special series with all the accuracy possible. Since temperature has no effect on the relation, as shown by Fig. 4, and since the duration of stimulus has no effect, provided it be not too short, as shown by Figs. 4 and 5, a temperature and a duration of stimulus were chosen to give the greatest accuracy of observation, with as little fatigue as possible. These observations were continued only to lengths 100 on the long side, since it had not been realised at the time how important the higher range would prove. Subsequent experiments therefore were carried out to complete the investigation over the higher range of lengths. The experiments at the shorter lengths were on muscles in Ringer's solution, with reverse but without calibration: those to the right, at the greater lengths, were in oxygen, with calibration but without reverse. In the case of such severe stretching as that administered to these latter muscles a permanent extension of a few millimetres is always produced, and a reverse series is valueless. Each experiment over the higher range consisted of five observations only, starting from length 100 and going to length 140 approximately. After this the muscle was killed and a calibration carried out. The observations at the greater lengths were then fitted to those at the lesser lengths by adjusting the scale to make the values at length 100 coincide. To be precise, the mean values of the heat—five means obtained at five different degrees of extension—were reduced to a constant maximum of 93, in order to make the first point coincide with the end of the curve obtained from the previous observation. In the same way, the mean tensions were reduced to a constant maximum of 99, in order to make the end of their curve coincide with the end of the curve given by the previous observation. That this process is justifiable is shown by the fact that the mean value of the tension found at length 110 in the first set of observations comes fairly accurately on to the curve of the second observations so determined. It is unfortunate that the importance of making the observations over the whole range was not realised earlier in the investigation: later a few experiments were made over the whole range and the results of Fig. 6 entirely confirmed. In some ways it is an advantage to split up the observations into separate experiments, since this avoids the effect of fatigue or damage, which in the case of the stretch experiments, causes rapid deterioration of the muscle.

Table I includes the data from which the left-hand side of Fig. 6 was drawn.

TABLE I. Frog's sartorii at 10° C.: 0.5 sec. maximal tetanus; heat in galvanometer scale divisions reduced to a constant sensitivity of galvanometer,  $4 \times 10^{-6}$  volts = 100 mm.

Length of muscle as p.c. of resting unloaded length.								
No.	110*	100	95	90	80	70	60	55
1	164	184	192	192	181	155	132	122
2	137	154	161	164	157	132	117	110
3	160	178	191	197	194	171	151	141
4	110	142	152	158	140	121	105	—
5	118	150	156	155	150	126	110	—
6	148	172	183	186	183	165	150	—
7	243	256	277	277	272	233	202	197
8	258	289	297	314	305	262	232	228
9	154	182	192	207	219	223	226	—
10	185	219	231	248	260	263	257	—

\* The numbers in this column do not strictly represent the heat, because of the absence of a calibration at length 110.

The results, after averaging, are shown in Fig. 6. Nos. 9 and 10, made on the same muscle, are anomalous, and are not included in the average. They represent the only example found in the whole of this investigation of heat production increasing continually with decreasing length. No explanation can be offered of the anomalous behaviour of this particular muscle, but the possibility of such an occurrence is of interest.

The fact that the tension developed varies with length has long been known. On the short side of the resting unloaded length this diminution of tension with shortening would occasion no surprise, being a general property of an elastic body. At a length of about 50, which may be regarded as the "natural" length of the excited muscle, the tension exerted disappears: at lengths greater than 100 the tension developed diminishes with extension. This is true of such simple muscles as the frog's sartorius or the tortoise's biceps cruris, in which the fibres are straight and parallel, and not supported against a load by tendons or connective tissue. In the case of a complex muscle such as the gastrocnemius the relation is presumably not so simple. Fig. 6 shows the striking symmetry of the tension-length curve, about a relative length of approximately 98. There is indeed a tendency to spread out to the right, which, however, may well be accounted for by the permanent extension set up by straining the muscle beyond a relative length of about 125. The curve indicates that at an extension of 50 to 60 p.c.—if the muscle or its tendon does not break—no tension will be developed on stimulation. This is almost literally correct.

The same type of symmetry is shown by the relation between heat and length, although the maximum heat production seems always to occur at a length slightly less than that of the maximum tension. With the heat also there appears to be a tendency to spread out to the right, but this again can be accounted for by the permanent extension pro-

duced in the muscle by stretching it beyond a length of about 125. It is striking that the factors, whatever they be, which determine the amount of energy liberated in a contraction, are affected to an equal degree by an extension and a shortening. The same symmetry will be found again in the very extensive set of observations on  $T/H$  shown graphically in Fig 7 below.

Another striking fact emphasised by the broken lines to the left of Figs 4 and 5 is that in many experiments the heat production, as it diminishes with diminishing length on the short side of length 100, appears to be aiming at the origin. The relation settles down to a simple proportion between heat production and length. It is tempting to imagine that if the muscle were able to overcome its own lateral rigidity, or whatever stops it from shortening further, the heat production would continue to diminish, tending to vanish at length zero. The series of observations recorded in Fig 6 is the only one which does not illustrate this proportionality: there the heat production diminishes rather less rapidly than does the length of the muscle. In general, however, there would seem, once the curve has passed its maximum and rounded the corner, to be a direct proportion between heat production and length, up to the stage when the curve ends, so to speak, in mid air because the muscle is unable to shorten any further.

Whether this proportionality represents any fundamental fact it is impossible at present to say. At first sight it might have been thought to indicate that the heat production is determined by the area of some surface running the full length of the muscle fibre. It is difficult, however, to imagine any kind of surface in the muscle which will diminish in direct proportion to its length. For example, in the case of a long thin cylinder of constant volume, *e.g.* a muscle fibre or fibril, the area of the surface is proportional, not to the length but to the square root of the length, and a constant proportion between heat and square root of length is definitely not the relation suggested by Figs 4 and 5. Moreover, if the heat were determined by the area of any such surface it would be difficult to explain why, as the length increases beyond 100, the heat production passes through a maximum and then decreases again. Some cause other than a simple proportionality to surface must be responsible for the relation between heat production and length.

That Heidenhain was correct in his statement that the heat production reaches a maximum at a certain length, beyond which any further extension results in a diminution of the heat, is very clearly shown by these experiments. The maximum heat production occurs at

a relative length of about 95. It is realised that the "resting unloaded length" of a muscle is a very arbitrary quantity. It is defined here simply as the full length of the muscle substance, not including any of the tendons, when the muscle is extended by a load of 1 to 2 gms. It cannot, however, be far from the length which the muscle occupies in the body.

The effect of stretching is presumably not upon the mechanism by which chemical energy is transformed into mechanical energy, but rather upon those "governors," whatever they be, which regulate the amount of energy expended. As Hartree and Hill(s) showed, in their studies of the heat produced in contractions of various durations, there is a very distinct regulatory mechanism determining the amount of energy liberated in a maximal continuous tetanus. There is, so to speak, a channel along which energy or chemical transformation must pass, and the size of this channel is very largely affected by a change of temperature. The chemical reactions which determine activity in the muscle are limited in extent, and the speed at which chemical change can occur rises as the temperature rises. In the phenomena described here we have found an influence of mechanical, one might even say geometrical, conditions upon the mechanism by which the supply of energy is regulated. In discussing—as in the next section of this paper—the relation between heat produced and tension developed we are dealing with the mechanism by which chemical energy is transformed into mechanical energy and work. Hitherto, however, in discussing simply the magnitude of the heat (or the tension) developed in a maximal contraction under various conditions, we are dealing not with the mechanism by which energy is transformed from one form to another, but with that by which the amount of energy so transformed is regulated: we are dealing, to take an engineering analogy, not with the cylinder, the piston and the valves, but with the governor which determines the amount of steam which is let into the cylinder. It is necessary to make this distinction clear, since otherwise false deductions might be drawn. The relation between heat production and tension, which is what gives us information about the other part of the machinery, is dealt with separately below.

It is not proposed in this paper to discuss certain obvious applications of the variation of heat production with length: it is sufficient to indicate that the diminished energy liberation associated with a shortened muscle must have a considerable bearing upon the economy of muscular movement in general, and that it is striking how the maximum energy

liberation for a given stimulus occurs nearly at the length at which the muscle lies in the body. If, as would seem probable, the same type of relation holds in the case of cardiac muscle, there are certain obvious applications to cardiology.

## II. THE RELATION BETWEEN LENGTH, AND THE RATIO OF TENSION DEVELOPED TO HEAT PRODUCED

In studying the actual mechanism of the muscular response the variation, with length, of  $T/H$  would seem to be of greater interest than that of  $H$  alone. If a muscle for any reason liberates more lactic acid when stimulated, we should expect it to give more heat and to develop more tension. In studying, therefore, the variation of  $H$  with length, we are never safe from the possibility that the effect observed, when the length is altered, is due to the "governor" mechanism discussed in the previous part of this paper. Moreover, in any kind of machine the most important factor in its working is that associated with its mechanical efficiency, the ratio of its mechanical output to the total energy it uses. In muscle for various reasons the maximum mechanical efficiency is difficult to determine. The simplest representation, however, of the response of muscle is the tension which it develops, and in many ways the most interesting and important criterion of a muscle's behaviour is the ratio of the tension developed to the heat produced. Since tension and heat are not quantities of the same dimensions, we must, if we wish to express  $T/H$  in absolute units, multiply the numerator by something of the dimension of length to make it of the same dimensions as the denominator. The obvious length to multiply it by is that of the resting unloaded muscle ( $l$ ). The quantity  $lT/H$  is one of considerable significance, being constant, in a twitch or a short tetanus, for variation of temperature and even for variation of the species from which the muscle is obtained. For such reasons, therefore, it was desirable to study the variation of  $T/H$ , or of  $lT/H$ , with length.

The experimental method was identical with that described in the previous part and the results are shown in Figs 7 and 8. Fig 7 is a composite one, as was Fig 6. The observations shown to the left were made on frog's muscle in Ringer's solution, uncalibrated, starting from length 100 and proceeding at intervals to the shortest length at which the muscle would develop any tension. Observations were made, as shown, at three temperatures and for three durations of stimulus. Many experiments were performed, the observations in each case when plotted lying upon a smooth curve. It was impossible, however, to draw

all these curves in one diagram, and since it seemed desirable to show the degree of variation found in individual experiments, it was thought best

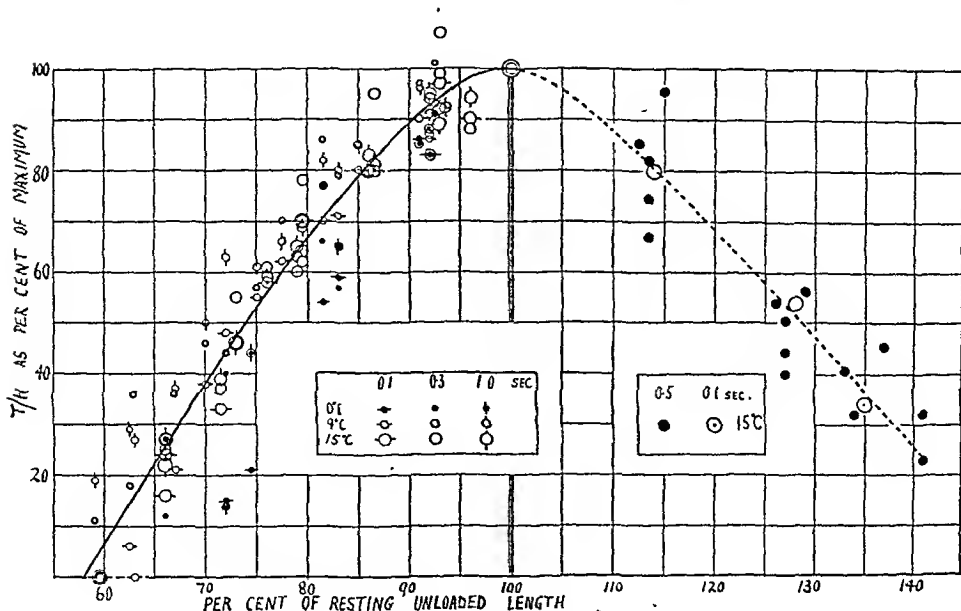


Fig. 7. Ratio of tension developed ( $T$ ) to heat produced ( $H$ ) as p.c. of maximum value of  $T/H$ , which in all except two of a very large number of cases was at 100 p.c. length. Observations to the left of the 100 p.c. length line made on *Rana temp.* at the temperatures and with the times of stimulation noted. The curves for the individual experiments were smooth, but since large numbers of curves cannot be shown in the same diagram, the observations only are given. Observations to the right of the 100 p.c. length line made on *Dutch Rana esc.* at  $15^{\circ}\text{C}$ ., 0.5 and 0.1 sec. tetanus, with calibration to allow for the varying sensitivity of the thermo-electric apparatus with varying length and thickness of muscle. Curves made to meet at the 100 p.c. point.

to include in one figure all the observations made. These observations may appear rather scattered, but it should be realised that the individual curves, of one of which each observation forms a part, are themselves smooth, and that the "scatter" shown is not due to experimental error but to individual variation of the muscles. This variation seems to be a random one except in one respect, namely, that the observations made at the lowest temperature, especially in the case of the shortest stimulus, all lie below the curve passing through the mean of the other observations. This fact would seem to be due to the cause discussed above (p. 246). The muscle at a low temperature moves so slowly and has so high a viscosity that during a short stimulation it has no time to develop its full tension at anything but the length 100. Apart from this

consistent difference, the heavy line drawn through the middle of the observations would appear to represent, with considerable accuracy, the average relation between  $T/H$  and length on the short side of the resting unloaded condition

The experiments shown to the right of length 100 required a calibration number at each length, and were made by the method described above. These observations were then fitted to those shown on the left by reducing them also to a constant maximum of 100 at the unloaded length. The double circle, therefore, which is shown at the point 100, represents one point in every set of observations made, whether to the right or to the left. In all series the value of  $T/H$  at length 100 is taken arbitrarily at this point. The observations to the right, that is those on the stretched muscle, were made at  $15^{\circ}\text{C}$  and for 0.5 and 0.1 sec of stimulation only. Since, however, the observations to the left show no difference between different temperatures and durations of stimulus, except for the one difference already mentioned, it is justifiable to continue the curve to the right by observations at one temperature only and with not so many durations of stimulation. The broken line drawn through the observations represents with fair accuracy the average relation between  $T/H$  and length for a stretched muscle.

Lest any error might arise in the construction of such a composite curve, a few experiments were carried out over the whole range on the muscles of Dutch *Rana* etc in good condition. Fig 8 represents two

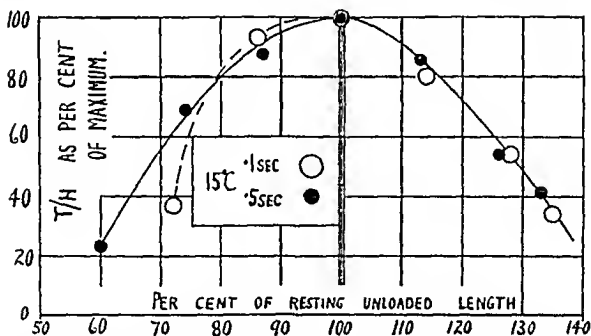


Fig 8 Two individual experiments, the last experiments made, relating  $T/H$  to length. In each experiment observations began at the shortest length, and were continued with 5 mm increments of length to the greatest. Note the similarity to Fig 7

such experiments. It is seen from Fig. 8 that individual muscles show identically the same kind of relation as that presented in the composite curve of Fig. 7 taken from a large number of different muscles. The symmetrical curve of Fig. 8, which is drawn through the full circles representing 0.5 sec. tetanus at 15° C., is exactly similar to the average curve of Fig. 7. The broken curve, drawn through the hollow circles, falls—on the left—below the full curve. In a contraction of such short duration the tension tends to be abolished by a smaller amount of shortening than in a contraction of greater duration. This fact we have already discussed, both here and in the preceding part. Details of one of the experiments shown in Fig. 8 are given in Part I above (p. 243).

There are several striking facts which emerge from Figs. 7 and 8. In the first place it is clear that the maximum value of  $T/H$  occurs almost exactly at what we have called here the "resting unloaded length." Only in two observations out of the very many shown in Fig. 7 was a greater value recorded than that at length 100. It is interesting to find that a muscle develops force with the greatest efficiency when at or near the length at which it lies at rest in the body.

A more striking fact, which we have discussed already in Part I in reference to other observations, is the almost exact symmetry of the curve. Here, as in Fig. 6, there is a tendency for the curve to spread out to the right at the greater extensions. This, however, can be explained if we remember that the large extensions leave a permanent mark on the muscle, so that it never returns precisely to the same resting unloaded length. A permanent stretch of 2 or 3 mm. is always found, and if this be allowed for it will make the curve almost exactly symmetrical. The shape of the curves indicates that at an extension of about 50 p.c. the value of  $T/H$  will fall to zero.

What the explanation of this relation can be it is impossible at present to say. It would seem, however, that this considerable variation of  $T/H$  with length, together with the symmetry of the relation and the fact that the maximum is at length 100, is a base line from which any theory of the mechanism of muscle must start.

It is not proposed to discuss here any possible application of these facts. It is sufficient to deduce that muscles, either extended or shortened from their natural unloaded length in the body, produce and maintain a tension with less economy than at that length. At extreme extensions or shortenings the economy becomes very poor indeed. Perhaps in the body this is, to some degree, counterbalanced by the greater mechanical advantage obtaining when a limb is partially flexed.



### III. THE MECHANICAL EFFICIENCY OF THE MUSCLE REGARDED AS AN ELASTIC BODY

The observations recorded here emphasise a difficulty which has been growing during the last few years in regarding the muscle as an elastic body whose elastic properties change as the result of stimulation. In Fig 9 is shown the relation between the tension developed and the

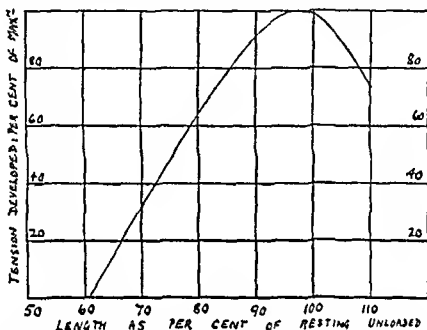


Fig 9 Relation between tension developed and length for the case of a short tetanus, 0.1 sec. Mean of six experiments at 9° and 15° C. Muscle in excellent condition in oxygenated Ringer. For calculation of the theoretical maximum work see text.

length, in an isometric contraction of a frog's sartorius muscle stimulated for 0.1 sec. Six experiments were performed, three at 9° C and three at 15° C, and the results were plotted and averaged as described above for the heat production. The muscles were in excellent condition, in oxygenated Ringer's solution, and the results of the six experiments were in good agreement. Experiments were made also at 0° C, but these differ from the others at the shorter lengths, presumably owing to the fact that muscle, because of its viscosity, never has time to attain the shorter length before its tension has begun to pass off. The actual means are not plotted, since they lie exactly on the curve shown. All the observations were reduced to a maximum of 100 before averaging, and, as before, it is seen that the maximum tension occurs at a relative length of about 98. At this length, therefore, the tension developed is taken arbitrarily to be 100. Now if the muscle could be regarded simply as an elastic body, the area of the curve would undoubtedly represent the maximum work which it could do. Even if the muscle in actual practice

could not perform this work, it might still be possible to argue that, owing to its internal viscosity, it could not shorten rapidly enough along the curve in time for the whole work to be done, before relaxation had caused the disappearance of its active state. Up to the time of Fenn's work (2) this view was indeed very generally accepted, and in 1913 I recorded experiments (9) in which the theoretical maximum work calculated from such a diagram was compared with the actual heat liberated by the muscle when subjected to a short stimulation. In this way a theoretical maximum for the mechanical efficiency of the initial process was determined, which reached the rather astonishing value of about 100 p.c. Such a value was of course possible, especially in view of the fact that the process considered was only part of the whole cycle, recovery providing an amount of heat somewhat larger than that liberated in the whole of the initial process. The experimental facts, however, which have been made available in the last few years, and especially the observations recorded in this paper on the variation of heat production with length, make it necessary to reopen the discussion.

From the diagram the area corresponding to the theoretical maximum work has been calculated at various lengths of the muscle, and the following values found:

Relative length	Ratio, $W/T_{100}l_{100}$
80	·063
90	·144
100	·244
110	·333

Here  $W$  implies the theoretical maximum work in a complete shortening from the length considered, and  $T_{100}$  the tension developed by the muscle at length  $l_{100}$ , the resting unloaded length. Now it is possible to pass directly from  $T_{100}l_{100}$  to the initial heat production, and so to find  $W/H$  in absolute units. The maximum value of  $Tl/H$  occurs, according to Figs. 7 and 8, at length 100. Here, according to a number of experiments made by Hartree and Hill (8) confirmed by many recent observations both on frog's sartorius and tortoise's biceps cruris, the value of  $Tl/H$  in a twitch, or a very short tetanus, is uniformly about 5. Thus we may write  $H_{100} = 0.2 T_{100}l_{100}$ , from which

$$W_{100}/H_{100} = 1.22.$$

In 1913 I found the theoretical maximum work to be about equal to the initial heat: the more accurate data now available make it on the average somewhat larger. Such a value as 1.22 for the ratio is not, in itself, impossible, since in the complete cycle the heat liberated in

recovery must be added, and this is 1.5 times the initial heat, so reducing the theoretical maximum efficiency to 0.49. It is instructive to repeat the calculation for length 110. Here we find

$$H_{110}/H_{100} = 1.66$$

From Fig. 6 we find

$$H_{110}/H_{100} = 843/93 = 91,$$

so that

$$W_{110}/H_{110} = 1.82$$

This value again is not in itself impossible, since for the whole cycle, including recovery, the theoretical maximum efficiency works out to 0.73. Moreover, if we could imagine the muscle shortening reversibly through the whole cycle it would have to pass through the various intervening lengths, and we might well suppose the heat liberated to be not  $H_{110}$  but the maximum heat, say  $H_{90}$  (Fig. 6). This would give

$$H_{100}/H_{90} = 1.13 \text{ (whole cycle 0.47),}$$

$$H_{110}/H_{90} = 1.54 \text{ (whole cycle 0.62)}$$

Even if we took the whole area, up to extreme excursions, of the tension-length curve, which from Fig. 6 is about double (actually rather less than double) that up to length 100, we should still find

$$H_{\text{total}}/H_{90} = 2.26 \text{ (whole cycle 0.90),}$$

which again is not an impossible value. The free energy of the oxidation of carbohydrate, on which muscular contraction is based, is almost certainly great enough to allow such values as 90 p.c. for the theoretical mechanical efficiency of the whole cycle. Hence physical chemistry does not prohibit, even in the extreme case, the use of the tension-length diagram as an indicator of the theoretical maximum work.

It is obvious nevertheless that the variation of heat production with length does introduce a very serious complication into the elastic body theory, just as do Fenn's results(2) on the extra heat production associated with mechanical work. In view of the demonstrable viscosity of the excited muscle it is not surprising that the actual work can never—even approximately—attain, within the finite time occupied by a twitch, the theoretical value calculable from the tension-length curve. If, however, we could imagine the active state "fixed" without continual stimulation, or if we could suppose the viscosity eliminated, might we even then believe the active muscle capable of passing, like an elastic body, through the whole cycle of its tension-length curve? In a state of tetanus it undoubtedly can, as Fick showed(10) long ago with his "Winkelhebel" with that device, as also with his "Schwunghebel" though to a less degree, he was able to obtain actual external work not far short of the area of the tension-length curve the same

fact may be shown for human muscles with the inertia ergometer, as used by Lupton<sup>(11)</sup>, Lindhard<sup>(12)</sup>, and myself<sup>(13)</sup>. In such experiments, however, the heat production is much greater, owing to the maintenance of the contraction: while the muscle, owing to its slow movement, can "adapt itself" to each new length as it arrives at it. It may well be the case that the fully tetanised muscle behaves simply as an elastic body possessing viscosity, though in it also the rate of energy expenditure required to maintain a contraction varies with the length. The muscle, however, which has been subjected to a short tetanus, or a single shock, possesses such a complex set of energy relations, as described by Fenn<sup>(2)</sup>, Azuma<sup>(5)</sup>, and in this paper, that we may well be forced to leave unanswered the question proposed above, can we even imagine the muscle stimulated with a single induction shock to be capable of passing through the whole cycle of its tension-length curve? If we can, then we are left with a series of perplexing questions: which heat ought we to compare with the work so obtained? the heat at the length of stimulation? the maximum heat at length 90 or thereabouts? the average heat over the whole range of shortening? and might we expect extra heat to be liberated, in accordance with the work done, as found by Fenn? Such questions can be answered only when we have a greater knowledge of the actual mechanism of contraction, a knowledge which perhaps may be afforded by the development of the theory which W. E. Garner describes elsewhere<sup>(14)</sup>.

#### IV. A POSSIBLE MECHANISM OF THE FENN EFFECT.

While, in many respects, the muscle behaves as an elastic body, there is obviously some mechanism in it adapting the amount of energy liberated to the work performed. The following thermodynamic conception may help perhaps to reconcile the two points of view.

Imagine a set of electrical accumulators providing current for a motor driving a heavy flywheel: allow the motor to do external work: disconnect the load, and then by suitable electrical connections, cause the kinetic energy of the flywheel to drive the motor as a dynamo and so to recharge the accumulators. Part, then, of the energy liberated by the discharge of the accumulators, the part not used up for doing mechanical work, is restored to them afterwards. In such a system there will be an extra total liberation of energy when work is done, since there will be correspondingly less kinetic energy to employ after work in recharging the accumulators.

When a muscle contracts, glycogen disappears and lactate is liberated. The lactate is reformed to glycogen afterwards, in the "recovery process," by means of free energy provided by the oxidation of carbohydrate or of lactic acid. The essential thermodynamic factor in recovery is the introduction of free energy, provided by oxidation, into a reaction, in order to drive it in the reverse direction from that in which it naturally goes. Thermodynamically speaking it is not necessary that this free energy should be derived from any special source: an electric current, whatever its origin, can liberate oxygen and hydrogen from water. Now in an actively contracting muscle there is a store of free energy, liberated in response to the stimulus, viz. the potential energy of the muscle as an elastic body. There may be some dispute as to the magnitude of this potential energy: there can be no doubt of its existence. The common view is that, in relaxation, the whole of this mechanical potential energy, if not previously employed in doing work, is dissipated into heat. Such a view, however, is not necessary, and one might well be led to look for a way in which the organism could avoid such an obviously uneconomical procedure. It would seem possible that part, at any rate, of the free energy of the muscle, while in a state of active tension, is used in commencing to carry out the recovery process which has later to be completed by oxidation.

The presence of such a mechanism would explain why extra energy is liberated, in shortening under a load, in proportion to the work done. The energy used to do external work is not available to assist recovery during relaxation, and the total breakdown is greater. It would explain also the converse phenomenon, viz. that less energy is liberated by the muscle when stretched during contraction: the extra potential energy provided by the stretch can cause a greater restoration during relaxation, and the total breakdown is smaller. It should not be impossible to devise an experimental test of this hypothesis.

#### SUMMARY.

1. Experiments are described by which the relation between heat production and length of muscle was determined, over a range of lengths from about 50 to 110 p.c. of that of the resting unloaded muscle. The same effects are found for all temperatures and for all durations of stimulus. The heat production is at a maximum, for a given duration of stimulus, at a relative length of about 90 p.c. On either side of this position it falls off considerably, along a curve which is approximately symmetrical about the vertical line through its maximum. In many

experiments, on the short side of the maximum, the heat production over a considerable range fell off in direct proportion to the length of the muscle. The relation is described also between tension developed and length. This relation also follows a symmetrical curve, with its maximum at a relative length of about 98 p.c. These positions of the maxima of heat production and tension, although near one another, are definitely not the same. At high initial extensions, of the order of 50 p.c. of the unloaded length, the heat is about 60 to 70 p.c. of its maximum value, while the tension has practically disappeared.

2. The variation is discussed of the relation between tension developed and heat produced, when the length of the muscle is varied. The ratio  $T/H$  has a maximum almost precisely at the resting unloaded length of the muscle, and falls on either side of that length along a symmetrical curve which reaches zero at about 40 to 50 p.c. of shortening or extension.

3. It is pointed out that the variation of heat production with length is determined by what may be called a "governor" mechanism in the muscle, which regulates the amount of energy liberated in response to a stimulus; while the variation of  $T/H$  with length depends upon the nature of the mechanism by which chemical energy is transformed into mechanical energy in the muscle.

4. The question is reopened of the validity of using the area of the tension-length curve, for a muscle stimulated with a shock or a short tetanus, as an indicator of the theoretical maximum work which the muscle could perform. Calling  $W$  the theoretical maximum work calculated from the  $T-l$  diagram, and  $H$  the heat production,  $W/H$  has at the resting unloaded length of the muscle and on the average a value of about 1.22. Starting from lengths greater than 100 p.c. still higher values of  $W/H$  are obtained.

5. The question is discussed, but left undecided, as to whether, in a muscle twitch, the area of the  $T-l$  curve is really an indicator of the maximum work which, in theory, the muscle might perform.

6. A mechanism is suggested of the Fenn effect, *i.e.* of the fact that more energy is liberated by a muscle when it does external work.

I am greatly indebted to Mr J. L. Parkinson for the skill and accuracy with which he has carried out the majority of the experiments described above; to the Scientific and Industrial Research Department for making Mr Parkinson's services available; and to the Royal Society for a grant to cover the expenses of the investigation.

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# OBSERVATIONS ON THE COMBINATION OF $\text{CO}_2$ IN THE BLOOD OF THE BULL FROG (*RANA CATESBIANA*).

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VERY little is known about the reaction and the  $\text{CO}_2$  combining power of frog's blood owing to the difficulty of getting sufficient blood from an ordinary frog. Having procured a number of large male frogs (*Rana catesbiana*) each of a weight between 600–700 grm. it seemed a favourable opportunity to obtain some data concerning the relations between the  $\text{CO}_2$  tension and the  $\text{CO}_2$  combining power and the  $\text{pH}$  respectively of the blood and further to attempt to find the physiological limits of the  $\text{CO}_2$  tensions in the circulating blood in order to get some notion concerning the physiological range of its  $\text{pH}$ . The latter question is very difficult to decide and we were unable to answer it completely owing to the limited number of animals at our disposal and the considerable difficulties which arise from the complicated conditions of the gas exchange in amphibia since they have both cutaneous and pulmonary respiration. The  $\text{CO}_2$  is removed chiefly through the skin so that the blood in lung capillaries comes into contact with a gas of a very low  $\text{CO}_2$  tension (Krogh<sup>(1)</sup>). Further, there is only one ventricle where the blood from the right and left auricles must be mixed to an extent as yet unknown.

The methods used for the  $\text{CO}_2$  dissociation curves were firstly Van Slyke's<sup>(2)</sup> method (constant volume apparatus) for the determination of the volume p.c. of  $\text{CO}_2$  in the blood, which had been saturated at  $15^\circ \text{C}$ . in a saturator (300 c.c.) of Barcroft's type with the desired gas mixtures, and, secondly, Haldane's method for the analysis of these mixtures after the withdrawal of the blood sample. The extracting chamber of the Van Slyke apparatus was the 50 c.c. type and for each determination 0.2 c.c. of blood was used. The frogs were narcotised with 25 p.c. urethane and the blood was obtained through a cannula tied into the left aorta. The blood was kept on ice during the time of the determinations and did not alter, concerning its  $\text{CO}_2$  combining power, to any appreciable extent during this time. As we observed that the last portions of the animal's blood, which were removed with a



small syringe were very much diluted as regards the hæmoglobin content, we then withdrew the blood in two portions, each of about 1 c.c. and determined the CO<sub>2</sub> dissociation curve of reduced blood separately in these two portions. In this particular case, however, the two curves were practically identical (see the three first double estimations of curve *R* in Fig 1), perhaps indicating that the hæmoglobin, which is about the half of the value of human blood (Haldane's hæmoglobinometer reading 52 for the first portion and about 20 for the last portions), does not play such a predominant part as a buffer as it does in mammals' blood (8).

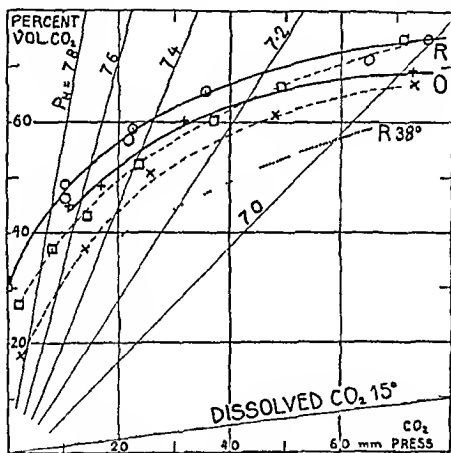


Fig 1

Fig 1 gives the CO<sub>2</sub> dissociation curve of the fully reduced (*R*) and the fully oxygenated (*O*) defibrinated blood of a male bull frog (continuous line) and of a male human individual (interrupted line) at 15° C and as a comparison the average curve of normal human blood at 38° C (3). The lines of constant pH in bicarbonate solutions at 15° C. are taken from Parsons (1).

The curves show that the absolute quantity of the CO<sub>2</sub> taken up at different CO<sub>2</sub> tensions (15° C) is greater than that in human blood at its physiological temperature and even somewhat greater than in human blood at 15° C, so that the frog's blood has a comparatively

high  $\text{CO}_2$  combining power. Using oxalated blood instead of defibrinated blood gives the same  $\text{CO}_2$  dissociation-curve.

Since it is as yet impossible to determine directly the actual hydrogen ion concentration in the venous or mixed blood of the frog, both  $\text{CO}_2$  tensions being unknown, the  $\text{pH}-\text{CO}_2$  pressure relation was determined in defibrinated blood taken as above. The hydrogen ion concentration was measured on reduced blood by means of the hydrogen electrode, as described by Parsons(5). A smaller form of the electrode vessel was used, so that 0.5 c.c. of blood was sufficient for each determination.

Fig. 2 shows a  $\text{pH}-\text{CO}_2$  pressure curve of the blood of two frogs at  $16^\circ\text{C}$ ., measured on different days. The shape of the curve reveals the fact that the blood is less well buffered than mammals' blood. This can be seen more clearly from Fig. 3, where (a) the relation of  $10^8 \times \text{cH}$  to  $\text{CO}_2$  tension (frog) is almost a straight line with a very slight concavity towards the abscissa. The slope of this line is steeper than that of the average line

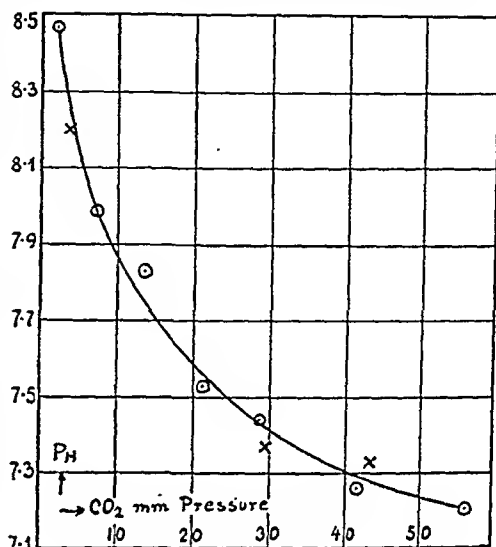
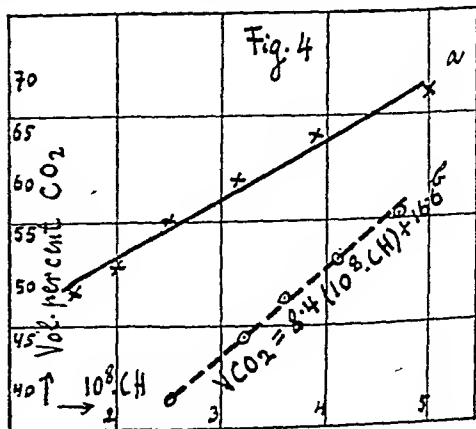
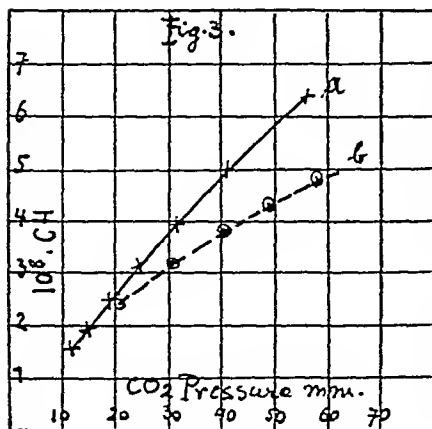


Fig. 2.



(b) for normal human blood(3) measured by the same method. This result is quite intelligible and one would even expect a more pronounced

difference, because frogs, with their low metabolism, are not in need of any more refined regulation of their blood reaction

Fig 1 pictures the volume p c CO<sub>2</sub>-10<sup>3</sup> × cH relation of frog's blood (a) and the average line for human blood (b)(3). It is possible to see from the relation between the total CO<sub>2</sub> (t CO<sub>2</sub>) and the 10<sup>3</sup> × cH the degree to which the blood is buffered. The general form of this relation is according to Baeroff and his co workers(3)

$$v \text{ CO}_2 = b (10^3 \text{ cH}) + c,$$

where *b* and *c* are constants. The absolute quantity taken up by the blood is determined by *c*, while *b* is a measure of the completeness with which the blood is buffered. The authors quoted give the value of *b* calculated from observations on eight individuals as  $8.4 \pm 2$  and *c* as  $16.6 \pm 1$ . Calculated from the observations in the frog's blood *c* has a value of 38.3 and *b* of 6.0, which is smaller than the lowest values found in the human blood (6.5). Considering also that the free CO<sub>2</sub> is much higher at 15° C than at 38° C, this value of *b* shows a less efficient buffering of the frog's blood.

The range of pH occurring in life might be roughly judged from the following data. Blood was withdrawn directly under mercury into the 0.2 c.c. pipette from different parts of the circulatory system and transferred immediately to the Van Slyke apparatus at 16° C

Blood from aorta	58.2	volume p c CO <sub>2</sub>	} Three different animals
" " cutaneous vein	62.0	" " "	
" " abdominal vein	70.1	" " "	

For aortic blood this would, according to Fig 1 a, correspond to a CO<sub>2</sub> tension of about 22 mm (reduced) and 29 mm (oxygenated), and these tensions again would (taken the average = 25.5 mm<sup>1</sup>) correspond on Fig 2 to a pH value of 7.18. One ought further to take into consideration that according to Evans(6) and others, the blood is more acid at lower than at higher temperatures, with a difference throughout of pH 0.2 for 20° C (38° and 18° respectively in human blood). This very approximate estimation only shows us the probable range of pH values in frog's blood. Rohde(7) published as the range of pH values of the blood of normal frogs (*Rana esculenta*, measured with Sørensen's hydrogen electrode improved by Bethe), pH 6.32-7.12<sup>2</sup>. These values are the more improbable because he paid no attention to the rôle of CO<sub>2</sub> in modifying the pH of the blood. In the method he used, the blood

<sup>1</sup> Supposing the haemoglobin is reduced to about the half in the mixed frog's blood

<sup>2</sup> He observed after feeding his frogs with boric acid a pH of 4.2 and after feeding them with sodium carbonate of 8.7 in the blood

must still have contained oxygen when it came into the hydrogen electrode, depolarising the electrode in this way to a variable extent, which lowers the potential difference, and the  $\text{CO}_2$  must have more or less disappeared, a proceeding which raises the  $p\text{H}$ .

#### SUMMARY.

The blood of *Rana catesbiana* (at  $15^\circ \text{C}$ .) binds a comparatively high amount of  $\text{CO}_2$  at different  $\text{CO}_2$  tensions, but is less well buffered than the blood of mammals. The reaction of the blood is under physiological conditions probably of the same order as that of mammals, though it may be slightly more alkaline.

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# AN ANALYSIS OF THE HEAT PRODUCTION DURING A CONTRACTION IN WHICH WORK IS PERFORMED.

By W. HARTREE<sup>1</sup>.

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RECENT experiments by Fenn(1, 2) have shown that when a muscle is allowed to shorten and do work there is a further output of energy beyond that liberated in an isometric contraction. When the load was held by the muscle during contraction only, and not during relaxation, the total extra energy liberated was approximately equal to the work done, so that the energy liberated as heat was constant. If the load rested on the muscle during relaxation then still more heat was given out. It seemed desirable to consider the matter in greater detail, and to make an attempt to follow the course of the heat production during the contraction by analysing the curve of galvanometer deflection using the method explained elsewhere(3). By such means it was hoped to determine in which phase the extra energy associated with work was liberated. With a view to this, and to other measurements of the kind, considerable improvements have been made in the technique.

The thermopile, constructed by soldering, has been improved by making it in one sheet, zig-zag fashion, holding each outer junction by thread to the supports, instead of winding the wire either round a sheet of insulator or round the arms of a U-shaped frame. If the same wire were used the new method would give only half the number of junctions per unit length, but by using finer wire (36 s.w.g. non and constantan) the number of junctions per unit length has been maintained (35 to 40 junctions per centimetre) without making the electrical resistance excessive. Using as little insulation as possible the thermal capacity of the part of the thermopile under the muscle is now much less than before, so that its temperature changes follow more quickly those of the muscle.

A further great improvement has been made by introducing a new suspension into the Paschen galvanometer used. This magnet system was constructed, with much stronger magnets of cobalt steel, by Mr A. C. Downing, of the Department of Physiology, University College,

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London. The figure of merit of the galvanometer has thus been increased three or four times, so that for a given sensitivity the deflection time is about halved. Either of these improvements means a closer correspondence between the temperature of the muscle at any moment and the simultaneous deflection of the galvanometer, and with both together the accuracy of analysis of the heat production has been largely increased.

It is perhaps worthy of note that the introduction of the stronger magnets has given rise to a considerable amount of electro-magnetic damping, due to the motion of these magnets setting up induced currents in the coils of the galvanometer. For this reason, when a resistance is added in series to diminish its sensitivity, the galvanometer must at the same time be shunted with such a resistance that the total resistance external to the galvanometer is constant. If this be not done the damping will be different for different resistances, in which case it has been found by experience that the analysis of the curve is not reliable.

Lastly a considerable improvement is the use of 50 volts instead of 25 volts to heat the muscle when making the control curves. This allows a much shorter time of heating to be used.

The muscle employed was the sartorius of *Rana temporaria*, which in all cases was stimulated for 0.1 second at 0° C. The lever carrying the load worked between two fixed stops, so arranged that the load was lifted 3 millimetres. Initially the loaded end of the lever rested on one stop, so that the muscle had a small initial tension. When the muscle had lifted the load through the full height, the lever, on touching the other stop, made the circuit of an electro-magnet which immediately held up the load. In a few cases the muscle was allowed to remain in its "short" position after the load had been held up, but it was then rather loose and its position on the thermopile uncertain. Better results were obtained by leaving on a net load of one or two grams, over and above that required to balance the weight of the wire connecting the muscle to the lever, so that when relaxation occurred the muscle was pulled back to its original "long" position on the thermopile.

There are certain obvious difficulties to be overcome and precautions taken. The accuracy of the analysis depends very much on that of the control curve, that is, on the curve of galvanometer deflection consequent on heating the muscle electrically for a very short time. It was obvious, however, that the control curve would be appreciably different in two cases in which the muscle was at very different extensions. It was necessary, therefore, to limit the experiment to the case of comparatively small shortenings; it was then found that if the muscle were

allowed to contract and to lift a load through 3 millimetres only, the control curves taken in the extreme positions, "long" and "short," corresponding to this shortening, were usually only very slightly different, say, by 0.5 p.c. of their maximum deflection at the most. Thus their mean could be relied upon for use in the analysis of the heat production when the muscle was allowed to shorten during the contraction. In all experiments it is to be understood that when the muscle did work the load was about the maximum which could be lifted the full height of 3 mm. Naturally more work could be done by allowing the muscle to lift a rather smaller weight through a considerably greater distance, and much more work by allowing the muscle to act on an inertia lever, or on a device similar to Fick's Winkelhebel(4). It was necessary, however, for the reasons given above, to limit the extent of the shortening, so that the simple isotonic contraction was restricted to a few millimetres, as in Fenn's experiments. The load, after having been lifted by the muscle, was always held up, in order to avoid the extra heat production associated, according to Fenn, with relaxing under a load.

Another reason for allowing only a comparatively small shortening, was that the principal object of the experiment lay in trying to find the difference in the course of the heat production in the two cases: (a) isometric contraction, and (b) muscle allowed to shorten and to do work. It was known that both the total heat production and also the time course of the heat production, during the contraction, were different for different initial extensions of the muscle. As the length of the muscle increases the total isometric heat increases also up to a maximum, and then falls off very appreciably at large initial extensions. In the latter case, moreover, the heat production is spread out over a longer time, as is also the mechanical response. If, however, care were taken to allow the 3 mm. shortening, when doing work, to occur in the neighbourhood of the initial length for which the isometric heat production was a maximum, then the magnitude of the isometric heat, and also its time course, were very similar in the "long" position and in the "short" position of the muscle. Working, therefore, in the neighbourhood of the maximum isometric heat, one could feel confident that any effect produced by shortening and doing work was not due merely to a change in the length of the muscle.

Twenty results were obtained from twelve different experiments. Each result came from a series of observations in the order: (1) isometric "long," (2) isometric "short," (3) lifting load, (4) isometric "short," (5) isometric "long," with two or three readings in each case. In this

way the effects of fatigue, if any, were eliminated. One reading in each position is insufficient, since the first reading after a change of circumstances is usually abnormal, a fact which must be due to the occurrence of some small physical, mechanical, or chemical "adaptation" of the muscle to the new circumstances.

When the muscle shortened, doing work, and relaxed unloaded, it was found, as shown by Fenn, that the isometric heat is indistinguishable in magnitude from the heat production when work was done. In these experiments the average difference was only 2 p.c., the isometric heat being that much the greater. When work is done, therefore, there is an extra output of energy (as distinguished from heat) over and above that liberated in the isometric contraction and this extra output of energy is equal to the work done. This result is a fortunate one for the present consideration, since now it is allowable to analyse the difference of the curves of galvanometer deflection in the two cases, namely, isometric and doing work, after reducing the curves to the same maximum, and so to find the difference in the course of the heat production in the two cases. The same result can, of course, be obtained by analysing each set of observations separately and comparing them, but greater accuracy is obtainable with less labour if the difference be analysed directly.

The results of the analysis in the twelve experiments referred to were very uniform, showing that when the load was lifted, about 15 p.c. more of the heat was produced in the initial 0.2 second of the contraction. Two only of the results lay outside the range 13 to 16 p.c. This period corresponds approximately to that during which the tension was rising in the isometric contraction. During this period the shortening muscle had further to do the work of lifting the load. This work had a mean value of 26 p.c. of the heat production, thus providing a mechanical efficiency of  $26/126 = 21$  p.c. in the initial phase, not including the recovery process. In the first 0.2 second, therefore, the total output of energy from the muscle when lifting the load was greater by about 41 p.c. of the actual heat production than when the contraction was isometric. During relaxation, however, the heat production in the shortened muscle is less than in the isometric muscle by 15 p.c. of the actual heat production. When a muscle shortens doing work extra energy is supplied equal to the work done, and in addition some of the relaxation heat is shifted into the first phase. It is natural that the relaxation heat should be less when work is done, since here the potential energy of the excited muscle has been in part turned into external work,



so one might expect less of it to be degraded into heat during the relaxation process.

The results of analysing the difference were verified by analysing separately also most of the cases of isometric contraction and of lifting load. This analysis gave the following for the average percentages of the actual heat appearing at different times:

Time in seconds	0	0.1	0.2	0.4	0.6	0.8	1.0	1.2
Lifting load	17	36	15	16	0	5	1	1
Isometric	17	26	10	22	15	7	2	1
Difference	0	10	5	-6	-6	-2	-1	0

It is satisfactory that the first number is the same in each case, since the heat liberated in the first 0.1 second must be due to the preliminary breakdown occurring in the muscle immediately on stimulation, and before the tension has risen appreciably. Its behaviour at any moment could not be expected to depend on actions occurring subsequently.

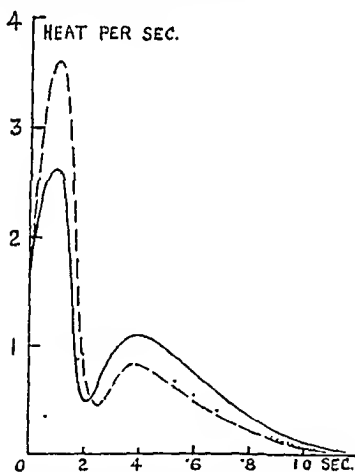


Fig 1 Rate of heat production in sartorius of *Rana temp.* 0.1 second tetanus at 0° C. Full line, isometric contraction, broken line, contraction when lifting maximum load a height of 3 mm. The unit of heat rate is the whole initial heat per second. Dotted line, an approximate isometric curve. The actual forms of the heat curves as drawn must be regarded as approximate before 0.1 second, especially in the neighbourhood of 0.2 second, as the analysis cannot be expected to give very accurate results when the heat rate is varying quickly.

The average results given in the above table are shown in Fig. 1, in which the similarity of the course of the heat production after 0.4 second seems to agree with the hypothesis that the difference between the two cases is due simply to relaxation from a greater tension in the isometric case, and the consequent degradation of a greater amount of potential energy. Fig. 1 shows the heat only, and not the work, so that the areas of the solid curve representing the isometric case and of the broken curve representing the case of work, are equal.

The numbers given in the above table for the heat liberated during an isometric contraction show that the course of the heat production is very different from that arrived at in the earlier investigations by Hartree and Hill(3). In view of recent improvements in the technique the results then obtained can now be regarded as little better than a first approximation. The full curve of Fig. 1 represents, so far as present knowledge goes, the time course of the evolution of heat in a frog's muscle stimulated with a tetanus lasting 0.1 second. A more accurate determination of the course of the heat production for different durations of stimulus is now in hand, and an account will be published shortly of a similar determination on the muscle of the tortoise.

In a few cases the muscle was given only a small load to lift through the usual 3 mm. In these cases the work done was about 10 p.c. of the maximum work obtained in the experiments already described. The results were not so uniform as before, but it was evident that there was still a large increase, from 10 to 15 p.c. approximately, in the heat production during the first 0.2 second as compared with the isometric case. The total heat production, however, was unaffected. The increase in the heat production during the first 0.2 second in this case suggests that the phenomena described are more concerned with the process of shortening in muscle, than with the actual doing of work.

#### SUMMARY.

Fenn's result has been confirmed that when a muscle shortens, doing work, and provided that the weight lifted is held up and does not bear on the muscle during relaxation, the energy liberated in the form of heat is constant, that is, it is the same as the heat liberated in an isometric contraction, and independent of the load. In such a contraction, therefore, the muscle supplies extra energy equal to the work done.

An analysis of the heat production in this case showed that, apart altogether from the liberation of this extra energy as work, there is a

considerable increase in the heat liberated during the earlier phases of contraction. This extra heat is compensated by a deficit during relaxation.

The simplest way of regarding the result is to suppose that when work has been done the potential energy available in the excited muscle is correspondingly reduced, so that there is less potential energy capable of degradation into heat during relaxation.

The expenses of this research have been borne in part by a grant from the Royal Society.

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# THE COAGULATION OF BLOOD PLASMA WHEN DIVESTED OF CORPUSCLES.

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THE experiments here described were devised for the investigation of three aspects of blood coagulation: (1) The rôle of the formed elements of the blood in that process. (2) The capacity of plasma to clot in the absence of all corpuscles. (3) The nature of the precipitate obtained by the prolonged cooling of plasma in an ice-chest (*i.e.* the "thrombozyme" of Nolf).

*Summary of earlier conclusions.* From the time of Bizzozero(1) to the present, blood platelets have been regarded as providing an essential factor in coagulation. Broadly speaking, two views are current: (1) That the lysis of platelets liberates a substance which inaugurates clotting (Morawitz(2), Fuld and Spiro(3), Mellanby(4), Bordet(5) and Stuber and Sano(6)). (2) That coagulation is caused primarily by the breaking up of platelets and secondarily by influencing the chemical reaction which takes place in the presence of the substance liberated from platelets (Cramer and Pringle(7)). Opposed to these views are the conclusions of Wooldridge(8) and of Nolf(9), who maintained that the plasma contains all that is necessary for its coagulation. A striking experiment by Cramer and Pringle(7), seemed to disprove this conclusion. Employing a Berkefeld filter, these observers removed the platelets from oxalated cat's blood and showed that it did not clot on re-calcification. The following facts indicate, however, that this experiment is not decisive: (a) oxalated blood after passage through a clay cell can be clotted by the addition of various proteoses and amino-acids (Zunz and Györgi(10)); (b) the acts of oxalation and re-calcification alter the condition of plasma (Pickering and Hewitt(11)); (c) "peptone" blood ultimately clots when platelets are absent (Achard and Aynaoud(12), Sacerdotti(13)). This is also true of blood after the injection of neutralised thymus nucleic acid (Pickering and Hewitt(14)), and of blood during pronounced thrombopenia (Roskam(15)). (d) There is no general correspondence between substances which are anti-lytic to platelets and those which inhibit blood coagulation (Pickering(16)).

In a preliminary communication to the Physiological Society, the present writers(17) pointed out that bird's plasma divested of all formed elements can be clotted by shaking, by prolonged contact with glass surfaces and by oxalation and re-calcification. The results given in the earlier part of this paper describe the continuation of this work.

Both Wooldridge(8) and Nolf(18) observed a deposit in the cooled mammalian plasma after the addition of anti-coagulants. Recently, Nolf(18) stated that the precipitate formed when the oxalated plasma of the horse is cooled at 0° C. for two days is the specific coagulant "thrombozyme" and that it is one of three plasma colloids which are essential for blood coagulation. Nolf also maintained that when the concentration of "thrombozyme" is diminished clotting is delayed. Mammalian plasmas were used in Nolf's experiments and from these it is not possible to remove completely all blood platelets. Proof was therefore lacking that the coagulant obtained was not contaminated with material derived from platelets. Too little material was available to permit of detailed investigation but it gave, however, the xanthoproteic, biuret and Millon's reactions. It was soluble at 37° in dilute sodium chloride and was re-precipitated on cooling to 0°. It was soluble in dilute alkalis and was re-precipitated on neutralisation. It was insoluble in alcohol and thus differed from the coagulant obtained by Bordet(5) and others from platelets. It does not appear to have been tested for phosphorus, which is present in all platelet extracts. In the latter part of this paper, the behaviour of the precipitate obtained by cooling pure bird's plasma for a period of from two days to one week to 0° C. will be described. In these experiments both the presence of the products of the lysis of platelets and the influence of oxalation are eliminated.

*Methods.* Blood was obtained from either the femoral or vertebral arteries of etherised domestic fowls. It was shed through paraffined cannulae into paraffined centrifuge tubes, externally cooled by ice. It was immediately centrifuged at 1000 r.p.m., the whole operation of collecting and centrifuging occupying 15 minutes. The plasma was then removed by pipettes into glass vessels. Both the pipettes and vessels were chemically cleaned and stored till used in a vacuum desiccator. The layer of plasma adjacent to the formed elements was discarded. Microscopic examination demonstrated the absence of all formed elements in the plasma. This was apparent both in the untreated plasma and when the stains of Leishman and Giemsa were used. Microscopic examination of the upper layers of the formed elements showed the presence of thrombocytes without any agglutination and the leucocytes were intact.

The thrombocyte and leucocyte counts of the whole blood when kept at 0° on a paraffined surface unexposed to the air remained constant during the time occupied by the preparation of the plasma.

To obtain the precipitate called "thrombozyme," the plasma after the removal of all formed elements was transferred to clean glass and the vessels sealed to prevent access of dust. It was kept for two days or longer at 0°, the deposit was isolated by the centrifuge, removed and washed.

*Results.* The plasma of the domestic fowl when divested of all formed elements exhibited the following characteristics:

1. It can be heated to 38° for four or five hours without clotting, but ultimately clots in 24 hours when in contact with glass surfaces. At 55° it becomes opalescent but does not coagulate. At 60° there is precipitation of presumably fibrinogen, as clotting cannot be produced after the plasma has been heated to that temperature.

2. It is resistant to the coagulant action of both the serum and the thrombin obtained from the blood of birds and of mammals. At 14·5° a clot was not formed till an hour had elapsed, when sufficient fowls' thrombin had been added to clot an equal volume of whole bird's blood in five minutes. At 40° this difference is less marked; the pure plasma clotting in eight minutes, the whole blood in two minutes. Parallel results were obtained when mammalian thrombin (Collingwood's preparation) was substituted for that of the bird.

3. When mixed with human blood the coagulation of the latter fluid is not accelerated and the clotting of the bird's plasma is not immediately induced. When a small amount of human blood is dropped into a watch glass nearly filled with pure bird's plasma the human blood clots whilst the bird's plasma remains fluid at room temperature for several hours. This result differs markedly from that arising from the mixture of whole bird's blood and human blood which completely clots in three or four minutes at room temperature. At the higher temperature of 40·5° the human blood clotted in one minute and the surrounding bird's plasma took three minutes. The presence of the human blood thus modifies the thermostability of the bird's plasma.

4. On the addition of sufficient 0·1 p.c. acetic acid to render the plasma neutral to litmus, it clotted at 16·5° in 43 minutes. At 40° a loose clot was formed in three minutes, which subsequently increased till a complete gel was formed a minute later.

5. After the addition of sufficient acetic acid to cause slight precipitation of protein (*i.e.* one-tenth of its volume of 0·1 p.c. acetic acid),

the material left in solution subsequently clots. Under these conditions a loose clot appeared in 20 minutes 30 seconds and a complete gel four minutes later.

6 Increase of alkalinity (by the addition of 0.1 p.c. potassium hydroxide) does not produce clotting.

7. When the plasma is kept in a sealed glass tube in an ice-chest for two days or longer it yields a precipitate which gives the following reactions: (a) Like the material obtained by Nolf, the precipitate is soluble in normal saline at 37° and is reprecipitated on cooling to 0°. (b) It is insoluble in alcohol. (c) It gives the xanthoproteic, biuret, tryptophane and Millon's reactions. (d) It contains phosphorus. (e) It is soluble in alkali and is precipitated again on neutralisation.

8 The plasma divested of the precipitate also contains small quantities of phosphorus.

9 The plasma after the removal of the precipitate ultimately clots both at room and body temperatures and thus behaves like whole plasma after the removal of all corpuscles.

10 The addition of the precipitate to the plasma from which the precipitate has been removed, accelerates the speed of clotting of that plasma. For example, the addition of the precipitate to the plasma caused clotting in 99 minutes, the control plasma remaining fluid. After heating the precipitate to temperatures from 100–120° for five minutes a similar coagulant activity was observed.

11 The addition of a mixture of thrombocytes and leucocytes to the pure plasma greatly hastens its clotting at 10°, coagulation being complete in a few minutes.

12 The addition of Merck's saponin in concentrations ranging from 1/12,800 to 1/51,200 to pure bird's plasma somewhat accelerated its speed of clotting at room temperatures (16.5–17° C). At the higher concentration clotting commenced in three hours and was complete in four hours. A control experiment showed that the pure plasma was fluid during this period. With concentrations of saponin ranging from 1/102,100 to 1/109,600 no alteration in the rate of coagulation was observed.

*Discussion.* The facts, we think, show that the blood plasma of the domestic fowl contains all that is necessary for its coagulation and that the participation of the formed elements of the blood is not essential for that process. The views of Wooldridge and of Nolf that blood coagulation is essentially an interaction of plasma constituents are thus confirmed. The coagulation of bird's blood divested of all formed ele-

ments is, however, much slower than when corpuscles are present. This difference is most marked at body temperatures, it having been shown that pure bird's blood clots at 38–40° in a few minutes (Pickering and Hewitt(19)), whilst the plasma divested of corpuscles takes 24 hours to clot. Blood platelets thus appear to be an important factor in the arrest of hæmorrhage, and the so-called "thrombokinese" of Morawitz to be an accelerator of coagulation rather than an essential factor in that process.

Although the alkalinity of bird's plasma tends to the preservation of its fluidity as suggested by Mellanby(4) and by Juan and Staub(20), it appears that the unneutralised plasma ultimately clots. A relative excess of hydroxyl ions is therefore not the sole cause of its stability. The hastening of clotting on neutralisation may be interpreted as due to the catalysing influence of hydrogen ions. Disturbance of the pure plasma inaugurates clotting as shown by the acceleration of that process produced by shaking, by partial precipitation with acetic acid, by oxalation and re-calcification and the addition of certain concentrations of saponin. The relatively slow action of saponin lends no support to the suggestion of Stuber and Sano(6) that the coagulant action of the "thrombokinese" of Morawitz is due to alterations in surface tension.

The material obtained by cooling pure bird's plasma to 0° exhibits all the chemical reactions of the "thrombozyme" of Nolf. It contains phosphorus. It is an accelerant of coagulation but its high thermostability points to its not being an enzyme. The analyses of plasma have hitherto been conducted with material contaminated with blood platelets and these formed elements are known to contain phosphatides. It is now shown that plasma divested of all formed elements also contains phosphorus.

#### SUMMARY.

1. The blood plasma of the domestic fowl collected in paraffined tubes and centrifugalised in the cold contains all the material essential for clotting. It is concluded that blood coagulation can occur without the intervention of either thrombocytes or tissue juices.

2. Various methods are described for producing the clotting of bird's pure plasma.

3. A slight increase of hydrion concentration tends to catalyse the clotting of bird's plasma.

4. Lowering the surface tension of this plasma increases its tendency to clot, but does not provoke immediate coagulation.

5. The precipitate obtained by cooling pure bird's plasma to 0°



exhibits the chemical reactions of the "thrombozyme" of Nolf. It contains phosphorus. It accelerates clotting but does not appear to be an enzyme, as implied in the terminology of Nolf, since it is not destroyed by heating to 100–120° C.

6 The plasma clots after the removal of this precipitate

7 Pure bird's plasma contains phosphorus

3

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## NOTE ON THE COMPOSITION OF ALVEOLAR AIR AT EXTREME HEIGHTS.

By T. HOWARD SOMERVELL.

DURING the Everest expedition of 1924 I made observations on the composition of alveolar air of several members of the party at three different heights above sea-level. The samples of air were collected in football bladders. In collecting a sample the subject first breathed out, held his breath for two seconds whilst putting the bladder to his lips, then expired as strongly as possible into it. This was done in exactly the same way in every case. It was not practicable to analyse at once the samples collected above the level of the base camp, on account of the freezing of the solution and other conditions. These, then, were taken to the base camp and analysed there; it will be seen from the analysis and considerations given below that there was no appreciable diffusion of gases through the wall of the bladder. I was unable to procure a Haldane apparatus in Calcutta, but by the kindness of Major Shorten, I.M.S. and his staff at the Calcutta Medical College, I managed to put together a similar apparatus of 150 c.c. capacity which gave consistent results in control experiments.

The air was collected by asking a man to breathe out, stop two seconds while putting the football bladder to his lips and gasp out into that. This was done in exactly the same way in every case in order that all results of analysis might be as far as possible comparable. Analysis was effected in the usual way by strong caustic soda for the  $\text{CO}_2$ , followed by the mixture of pyrogallic acid, exactly as in a Haldane apparatus, except that a longer time was allowed for the absorption of the oxygen. In each case a little water was left above the mercury. The gas burette was kept at an even temperature by the constant pouring of water at a fixed temperature over it. At the Base Camp this was easily managed owing to the presence of a spring.

At Pbari Dzong, the first series of observations was taken.

TABLE I. Altitude 14,300 feet. In each case 140 c.c. of alveolar air were analysed  
Barometer taken as 457 mm. Aq. vapour in lung as 47 mm.

Name	CO <sub>2</sub>		O <sub>2</sub>		Alveolar resp. quot.
	P c.	Partial pressure	P.c.	Partial pressure	
Norton	7.6	31	13.9	57	1.07
Beetham	6.9	28	11.8	48	.74
Somervell (1)	6.8	28	13.2	54	.87
(2)	8.2	34	9.8	40	.74
Irvine	6.9	28	11.7	48	.74
Shebbicare	5.2	21	14.6	60	.81
G. Bruce	7.3	30	12.6	52	—
Average	6.76	28	—	—	—

Another series at the base camp, three weeks later, when all were partially acclimatised, is as follows:

TABLE II. 16,500 feet above sea-level. Barometer taken as 425 mm.  
Aq. vapour in lung as 47 mm.

Irvine	5.1	19	15.3	58	.91
Mallory	6.1	23	14.6	55	.97
Beetham	6.0	23	14.0	53	.94
Somervell	5.3	20	12.7	48	.64
Norton	5.3	20	14.7	55	.82
Atmospheric air	.3	—	19.5	—	—

TABLE III. 23,000 feet above sea-level. Barometer taken as 330 mm.  
Aq. vapour in lung as 47 mm.

Mallory	2.5	7	13.6	39	.33
Irvine	2.7	8	12.7	37	.35
Norton	2.9	8	13.8	39	.39
	3.0	9	13.6	39	.43
Somervell	2.7	8	13.4	39	.39
	2.4	7	12.9	37	.31
	3.0	9	13.3	38	.44

TABLE IV. Controls at Base Camp, 16,500 feet above sea level subsequent to ascent.  
Barometer taken as 425 mm. Aq. vapour in lung 47 mm.

Air	3	—	20.6	—	—
Somervell	5.6	22	14.2	54	.88
Air	3	—	19.6	—	—
Norton	4.9	18	14.9	56	.69

The most remarkable point about the above figures is the low respiratory quotient at 23,000 feet. This was caused by an extremely small value for the CO<sub>2</sub> in the alveolar air. With a normal respiratory quotient the combined percentages of oxygen and CO<sub>2</sub> in the alveolar air should add up to nearly 20 p.c. of the dry air. At all stations except 23,000 feet this was the case.

TABLE V.

Altitude	Average p.c. CO <sub>2</sub>	Average p.c. O <sub>2</sub>	Total p.c. CO <sub>2</sub> + O <sub>2</sub>
14,300	6.76	12.31	19.07
16,000	5.58	14.17	19.75
23,000	2.78	13.59	16.37
16,000 on return	5.25	14.55	19.80

The controls on the way down were done because the total CO<sub>2</sub> + O<sub>2</sub> was obviously less than would have been expected, in order to ascertain that there was nothing the matter with the apparatus. They seem to indicate that the low value for the R.Q. at 23,000 was not due to gross error in technique, although the air analyses show a tendency for the oxygen readings of atmospheric air to fall somewhat short of the theoretical value. In calculating the respiratory quotient the theoretical value has been used. Had the average value given by the apparatus for the oxygen in air been used the respiratory quotient would have come out a little higher, but the difference would only be one of detail.

The fact that the apparatus gives rather low results for atmospheric air suggests that the alveolar oxygens as given are not too high.

At a great height breathing is so rapid (about 50 respirations to the minute) that the CO<sub>2</sub> is washed quickly out of the alveoli and hence in this series the percentage of CO<sub>2</sub> is naturally small: but I fail to see why the CO<sub>2</sub> + O<sub>2</sub> is also so small: yet there seem to be no gross errors in analysis, partly for the reason given and partly because the figures at 23,000 feet are very consistent.

The question of diffusion from the football bladders will not explain it, as nitrogen will diffuse slightly more quickly than CO<sub>2</sub> or O<sub>2</sub>, and if diffusion occurred the CO<sub>2</sub> + O<sub>2</sub> percentage should show a very slight increase if anything; personally I feel satisfied that at a great height such as 23,000 feet the (CO<sub>2</sub> + O<sub>2</sub>) percentage is undoubtedly smaller by some 3 p.c. than at less elevations such as 14,000–16,000 feet.

It may be of interest to record one or two personal observations which I made while climbing in the neighbourhood of 27,000–28,000 feet.

*Pulse.* The heart during actual motion upwards was found to be beating 160–180 per minute, sometimes even more; regular in rhythm and of good volume. All who had gone above 27,000 feet were found by Major Hingston, I.M.S. (the official doctor of the Expedition) to have dilated hearts, which took one to three weeks to recover.

*Respiration.* About 50–55 per minute while climbing. Approaching 28,000 feet, I found that for every single step forward and upward, seven to ten complete respirations were required. Breathing quickly

and deeply is very easy at a great height owing to the low density of the air.

*Mentality.* While the simplest little bit of extra work, such as cooking a meal, fetching snow for melting, or even taking a photograph, is very irksome, yet in 1924 I should say that we were none of us affected by the altitude mentally to the same degree as in 1922; our minds were clear and our tempers and resolution both fairly good even at a height of 28,000 feet.

*Appetite.* At our highest camp, 26,700 feet, the appetites of Colonel Norton and myself were both profoundly affected; we could with difficulty bring ourselves to eat meat at all; chocolate and biscuits were managed as a duty, and only for pemmican soup and coffee did we show any real relish: liquids in this high, dry air are, of course, one's primary need.

*Colour index.* (Hæmoglobin value.) At the Base Camp, May 2, the colour indices of various members of the party were:

116, 120, 122, 114, 116, 142, 126, 118.

At 21,000 feet, May 25, they were respectively

120, 120, —, 116, —, 136, 112, —.

The extra height and three weeks' acclimatisation had not apparently affected the colour index at all. Blood-pressure seems to be unaffected by altitude. The colour indices of two Tibetans, taken at 16,500 feet, at which height most of their lives had been spent, were 92 and 82; remarkably low figures for men who can race up steep slopes about twice as fast as we could with our colour indices of 120.

A STUDY OF THE MECHANISM OF INSULIN. Part I.  
The action of insulin and of the salts of guanidine on the  
permeability of the mammalian erythrocyte. By J. SECKER.

*(From the Department of Physiology, University of Durham.)*

THE means whereby insulin lowers the level of the sugar in the blood has been the subject of a large amount of work but is by no means understood. Eadie, Macleod and Noble(1) have shown that the fall in plasma glucose is not due to any action of the insulin on the glucose itself. Winter and Smith(2) believe that glucose is converted into a more active form by the action of insulin, this active form of glucose being more readily oxidised in the tissues. That increased oxidation of glucose is not the main mode of action of insulin seems to be shown by Burn and Dale(3). These workers observed that under the influence of insulin the output of carbon dioxide by the heart, during perfusion, does not increase in proportion to the amount of glucose taken up from the perfusion fluid. It seems justifiable to infer from this series of experiments that, whatever the ultimate fate of glucose under the influence of insulin, immediate and complete oxidation in the tissues does not occur.

Haldane, Kay and Smith(4) as a result of an investigation into the effect of insulin on the blood volume, suggest that definite changes in the permeability of certain of the body cells may be brought about by the action of insulin. This opinion, however, was not supported by any conclusive experimental evidence. The following experiments were planned to test the hypothesis of altered permeability. The influence of the salts of guanidine on permeability was considered in conjunction with the influence of insulin because certain experiments by Watanabe(5), Clark(6) and Burns(7) were suggestive of a close relation between these two substances. Further, it has been shown that during the period of development of the hens' egg, Burns(8), and during the germination of *Vicia* seeds, Schulze(9), at a time when increased permeability to building matter and to glucose is essential, the guanidine content of the organism is increased. The cell chosen for the present study was the mammalian erythrocyte since it acts as a semipermeable membrane to solutions of glucose and of sodium chloride.

*The influence of insulin on the erythrocytes*

*The distribution of glucose in plasma and corpuscles* The estimation of glucose was carried out by means of Benedict's colorimetric method (10) using a Kober colorimeter.

(a) For the first experiments freshly oxalated blood was obtained and, in every case, the tests were commenced within half an hour of the animal being killed. The sample of blood was divided into several parts and treated according to the tests to be done.

In a typical experiment (1) a sample was measured and set aside in the pierie pierate mixture, (2) a sample centrifuged to obtain plasma, (3) insulin, 0.2 c.c. B. D. H. per 50 c.c. of blood added, (4) a small quantity of glucose added (amount varied in different experiments), (5) insulin, 0.2 c.c. per 50 c.c. of blood, added to a sample of the blood to which glucose had been added. The samples 2-5 after mixing, and allowing to stand for 10 minutes, were centrifuged, 2 c.c. of the plasma from each being taken for a test. The blood or plasma was allowed to stand overnight in the pierie pierate mixture in the dark and the estimation completed on the following day. After consideration of the very great difficulties to be overcome in obtaining washed corpuscles for the estimation of glucose, and the great possibility of error entailed, it was thought sufficient to estimate the change in the glucose content of whole blood and of plasma before and after treatment with insulin, the glucose lost from the plasma being considered to have been taken up by the corpuscles.

(b) The tests were repeated, using defibrinated blood, the various samples being prepared in the manner described for oxalated blood.

The results given in Table I show a fall in the glucose content of the plasma, or serum, after treatment of the blood with insulin. The addition of insulin was without effect on the total glucose content of the blood.

TABLE I Influence of insulin on the distribution of glucose  
(The amount of glucose is given in mgms. per 100 c.c. of fluid.)

Oxalated blood	Whole blood	Plasma	Percentage fall
			in plasma after insulin
Sheep	62	58	7
"	72	78	6
Glucose added	—	404	10
			Percentage fall
Defibrinated blood	Whole blood	Serum	in serum after insulin
			Ox
			110
			129
Sheep	108	130	19
			11
Calf	148	177	13

*The distribution of chloride in the plasma and corpuscles.* The estimation of chloride was carried out by means of the method of Austin and Van Slyke (11).

(a) Using oxalated blood. Samples were prepared for estimation in the manner adopted for the determination of glucose, 3 c.c. samples of blood or plasma being used for each estimation. After precipitation of the chloride with silver nitrate, the mixtures were allowed to stand overnight in the dark.

(b) The influence of insulin on the distribution of chloride in defibrinated blood was also studied.

The results given in Table II show that, in all cases, whether oxalated blood or defibrinated blood has been used, there is a fall in the chloride content of the plasma after treatment with insulin.

TABLE II. Influence of insulin on the distribution of chloride.  
(The amount of chloride is given in mgrms. per 100 c.c. of fluid.)

		Whole blood	Plasma	Percentage fall in plasma after insulin
Oxalated blood.				
	Sheep	524	820	93
Defibrinated blood.				
	Ox	706	850	118
NaCl added.	„	2052	2506	316

*The influence of guanidine salts on the erythrocytes.*

*The distribution of glucose in plasma and corpuscles.* The glucose contents of oxalated blood, oxalated plasma, defibrinated blood and serum were determined by the method used in the study of the effect of insulin. Samples were prepared for estimation as above, 0.1 gm. guanidine carbonate being added in place of the insulin.

The results given in Table III show that, whereas there is a fall in the plasma glucose of oxalated blood, there is no such fall in the case of defibrinated blood. Guanidine was without effect on the total glucose content of the blood.

The results obtained with defibrinated blood show that some factor essential for the action of guanidine is missing from defibrinated blood. Defibrinated blood differs from oxalated blood in two respects, lack of fibrinogen and lack of that moiety of calcium necessary to induce the formation of fibrin. In view of the fact that calcium metabolism is seriously distributed in guanidine intoxication, leading to a distinct alteration in the state of the blood-calcium involved in the clotting



process, it was decided to try the effect of the addition of calcium salts to the defibrinated blood. For this purpose a small amount of calcium lactate or calcium chloride (0.1 p.c.) was added to the blood. Samples were prepared by the method described above. After the first experiment on defibrinated blood these tests were usually carried out in conjunction with those described above. The results of these tests on added calcium, given in Table III, show that the presence of calcium is essential for the action of guanidine in lowering the glucose content of the plasma. Further, the fall in serum glucose is usually greater than that obtained with oxalated blood.

TABLE III Influence of guanidine on the distribution of glucose

		Whole blood	Plasma	Percentage fall in plasma after guanidine
Oxalated blood	Cat (pregnant)	198	280	32
	Sheep	72	78	5
Glucose added	Sheep	—	133	8
	"	—	404	34
Defibrinated blood	Sheep	108	130	0
	"	92	100	0
	Calf	148	177	0
Glucose added	Ox	—	210	0
	"	242	344	0
Defibrinated blood to which calcium has been added				
	Ox	—	129	34
	Sheep	108	130	20
	Calf	148	177	15
Glucose added	Ox	242	344	20
	Sheep	—	210	24
		—	362	44

*The distribution of chloride in plasma and corpuscles.* The chloride contents of oxalated blood, oxalated plasma, defibrinated blood and serum, were determined by the method used in the study of the effect of insulin, 0.1 gm. guanidine carbonate being used in place of the insulin.

The results obtained showed again the insufficiency of guanidine alone to lower the chloride content of serum of *defibrinated* blood. Samples were treated as above with calcium salts and the fall of serum chloride obtained. The results are given in Table IV.

TABLE IV. Influence of guanidine on the distribution of chloride.

		Whole blood	Plasma	Percentage fall in plasma after guanidine
Oxalated blood.				
	Sheep	492	615	0
	"	524	820	92
NaCl added.	"	2097	2900	83
	"	1440	1713	68
Defibrinated blood.				
	Sheep	553	739	0
	Ox	500	627	0
NaCl added.	Sheep	1464	1820	0
	"	1453	1809	0
Defibrinated blood to which calcium has been added.				
	Ox	500	627	39
	"	706	850	100
NaCl added.	Sheep	2052	2506	205

Similar tests were carried out to show the action of dimethylguanidine sulphate on the glucose and chloride contents of blood and similar results obtained.

### *Microscopic changes.*

Evidence in favour of the view that insulin and guanidine have the power of increasing the permeability of the cell membrane is obtained from the results of the following experiments.

*Action of insulin.* Drops of blood from a finger were mixed with solutions of varying osmotic pressure and examined microscopically. The solutions used were NaCl 1·7, 1·5, ·09, 0·5, and 0·2 p.c., each solution being paired with one of the same strength to which ·25 c.c. insulin (B. D. H.) per 100 c.c. had been added.

The results were: (1) The corpuscles in the 0·9 p.c. NaCl retained their normal shape and size, whereas those in the corresponding insulin solution became crenated, then assumed a smooth spherical form having a smaller diameter than that of the normal corpuscle. (2) The corpuscles in the hypotonic solutions of NaCl swelled up and in the lower osmotic solutions became hæmolyzed, whereas those in the insulin solutions assumed the spherical form but did not show any appreciable increase in volume. In the more dilute solutions the corpuscles gradually faded from view, the hæmoglobin diffusing from the cell without any apparent rupture of the membrane due to swelling, ghosts of the cells remaining. (3) In the hypertonic solutions of NaCl the corpuscles became crenated while those in the corresponding insulin solutions took on the spherical form.

*Action of guanidine.* The method of comparing the erythrocytes in

solutions of equal osmotic pressures, described above, was used. The guanidine solution of each pair contained 0.1 p.c. of guanidine carbonate in place of an equivalent osmotic amount of NaCl.

The results of these experiments were similar to those described above for the action of insulin, excepting that the change from discoidal to spherical form occurred more rapidly. The tests on the action of guanidine were repeated using (1) solutions of glucose of the same osmotic values containing 0.1 p.c. guanidine carbonate, (2) solutions containing 0.1 p.c. guanidine sulphate in place of the carbonate, (3) corpuscles previously treated with 5 p.c. formalin. In all cases, excepting after treatment with formalin, results identical with those described above were obtained. After treatment with formalin no change in the form of the corpuscle was observed. Dilution of the blood with plasma or serum containing 0.1 p.c. guanidine carbonate failed to give any change in the form of the corpuscles.

The action of guanidine salts is similar to that of saponin as described by Ponder (12), whose account I can confirm, except that with saponin, hæmolysis follows immediately after the change in form of the corpuscles, whilst with guanidine the change in form is not followed by hæmolysis, at least not for several hours. The change of form of the corpuscles under the influence of insulin and guanidine does not appear to be due to mere dilution of the plasma, since addition of 0.9 p.c. NaCl alone does not cause the change.

#### *Discussion.*

The facts given above I consider show that insulin increases the permeability of the mammalian erythrocyte to glucose and chlorides, substances to which they (on present evidence) are impermeable or only slightly permeable. Thus insulin may be regarded as enabling those substances which are in relative excess in the plasma or in the corpuscles to pass into the corpuscles or plasma respectively. It is not unjustifiable to assume that insulin has a similar action on the permeability of the tissue cells, in which case the fall of blood sugar without a concomitant increase in sugar oxidation observable after the injection of insulin may be due to the passage of sugar (plus some nitrogenous body) into the tissues, especially muscle.

The facts also show that guanidine has a similar action to insulin provided calcium is present. Since excision of the parathyroids causes an increase of the guanidines and a decrease of the calcium present in the blood, the facts suggest a method by which the parathyroids may

influence carbohydrate metabolism. Further if, as Burns finds (unpublished experiments), insulin causes a liberation of guanidine, the guanidine with the existing calcium will reinforce the insulin hypoglycaemia.

#### SUMMARY.

1. Quantitative chemical and microscopic evidence is given in support of the view that the mechanism of insulin is to increase the permeability of the cell membrane.

2. Guanidine resembles insulin in its action of increasing permeability.

3. The presence of calcium is essential for this action of guanidine.

In conclusion I wish to express my gratitude to Prof. D. Burns for his help and criticism during the course of this research.

The expenses of this work were defrayed by a grant from the Medical Research Council for which I desire to express my thanks.

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# THE REDUCED SENSITIVITY TO INSULIN OF RATS AND MICE FED ON A CARBOHYDRATE-FREE, EXCESS-FAT DIET. BY H W. BAINBRIDGE.

(From the Wellcome Physiological Research Laboratories )

THE purpose of these experiments was to discover whether the sensitivity of animals to insulin could be altered by increasing the proportion of fat in the diet.

*Method* In the first series of experiments on rats the animals used were divided into two groups. One group was fed on the diet of Halliburton and Drummond (called the complete diet), the other group on an exactly similar diet except that the starch was replaced by butter (the excess-fat carbohydrate-free diet) the two diets being of approximately equivalent calorie value (Table I).

TABLE I Diets.

	Excess carbohydrate fat free grams	Complete grams	Excess fat carbohydrate free grams
Casem	18	18	18
Butter	—	20	40.7
Starch	91	47	—
Salt mixture	5	5	5
Marmite	5	5	5

The rats were kept in cages—two or three in each cage—provided with water and as much food as they could eat. At the end of a period, which varied from two to six weeks in the different experiments, the rats, which were starved for about 20 hours before injection, were placed in a room at a temperature of approximately 33° C. and injected subcutaneously with insulin. The dose given in all the experiments on rats was four clinical units per kilogram rat. Three clinical units per kgm. brings the blood sugar of 60 p. c. of rabbits down to 0.15 p. c. at room temperature. Rats are evidently normally less sensitive to insulin than rabbits. A room temperature of 33° C. was used because it has been found in these laboratories (Prevan and Boock, private communication) that rats are more susceptible to insulin, and show less range of variability at the higher temperature. Krogh (using mice) was the first

to notice that the action of insulin was much affected by the temperature at which the animals were kept. They were, therefore, placed in a room at this temperature a few minutes before injection and kept there afterwards for a definite period, usually two hours.

*Results.* Table II is a detailed record of two experiments made on growing rats of about 100 grms. weight which had previously been fed on ordinary laboratory diet (bread, milk and green food), and were transferred to the experimental diet six weeks before the date of these experiments. The symptoms, characteristic of insulin, were severe unless stated to be otherwise. Convulsive movements appear to be less usual in rats than extreme prostration.

The animals were kept in sets of three or four in separate cages and their body weights and sex noted. These details are omitted in the accounts of the experiments, since they were not associated with any difference in result.

TABLE II.

Diet	Rat	Time after injection at which symptoms became severe	
Complete diet	1	45 mins.	Dead in 1 hr.
	2	Convulsions 35 mins.	Glucose
	3	35 mins.	Dead in 1 hr.
	4	None	
	5	35 mins.	Dead in 1 hr.
	6	55 mins.	Dead in 1 hr.
	7	2 hrs.	Recovered without glucose
	8	2 hrs.	Glucose
	9	1 hr. 25 mins.	Convulsions 1 hr. 35 mins.
Carbohydrate-free excess-fat diet	10	1 hr. 40 mins.	Dead 1 hr. 55 min.
	11	1 hr. 40 mins.	Recovered in cold 1 hr. 55 mins.
	12	1 hr.	Recovered in cold 1 hr. 20 mins.

Six other rats on the carbohydrate-free excess-fat diet were similarly treated and insulin injection caused no symptoms.

In most of these cases one or more injections of insulin had been made during this period of six weeks without producing any obvious symptoms, the dose given (about a quarter of that finally adopted) being apparently too small to be effective. When rats 1-3 and 10-12 were tested the temperature was allowed to rise to 37° C., and this may possibly account for the fact that it was the only case in which all the rats on the excess-fat carbohydrate-free diet showed symptoms. Even here the onset of the symptoms was delayed in the fat-dieted rats.

The fact that rats fed on an excess-fat diet are less susceptible to the action of insulin than those on the complete diet is confirmed by further experiments, a few of which are summarised in Table III. These rats were given the special diet as soon as they were old enough to be taken from their mother, and were kept on it for a period varying from

2-6 weeks before injection No previous injection of insulin had been given

TABLE III

Diet	Rats	Duration of Exp	Symptoms
Complete	19-21	1 hr	All in 35 mins, one died later
	22-24*	2 hrs	2 in 1 hr 20 mins, 1 in 30 mins
	25-27	3 to 4 hrs	2 in 1 hr 20 mins, 1 in 20 mins All died later
	28 and 29	3 to 4 hrs	Both in 1 hr 40 mins Both died later
Excess fat carbohydrate free diet	30-32	1 hr	None
	33-35*	2 hrs	One slight symptoms, left in warm room, no worse in 2 hrs
	36-38	3 to 4 hrs	None
	39 and 40	3 to 4 hrs	One showed symptoms 2 hrs Convulsions 2 hrs 45 mins

\* These rats received 0.1 c.c. cod liver oil each per day for one week before injection

In the cases in which the rats on the carbohydrate free excess-fat diet developed symptoms, these symptoms were delayed and were less severe than those of the rats on the complete diet. Thus rat 22 on the complete diet exhibited convulsive symptoms half an hour after injection. These symptoms became so severe after twenty more minutes in the incubator, that even though it was removed to room temperature and given 5 c.c. of 50 p.c. glucose, it was dead half an hour later. The other two rats in this experiment (rats 23 and 24) showed severe symptoms 1 hr and 20 mins after injection and were dead ten minutes later. The effect of an equal dose of insulin on rats fed on an excess fat diet is very different. Only one of the rats (rat 33) injected at the same time showed symptoms which developed 1 hr 10 mins after injection. They were much less severe than those of the complete diet rats and had not increased in severity at the end of the experiment twenty minutes later. This rat was removed to room temperature and given 1 c.c. of 50 p.c. glucose—it was able to walk about immediately and was apparently perfectly well next day.

It is well known that animals vary widely in their response to insulin and these results might be due to the fact that the rats on the excess fat diet chanced to be among the extremely refractory group. To exclude this the following experiment was made.

(a) Seven rats after being fed on the complete diet for 13 days were given insulin. Two of these developed severe symptoms in 1 hr 5 mins, three in 1 hr 30 mins to 2 hrs and one had no symptoms. They were then fed on excess fat carbohydrate free diet for 13 days and on giving insulin, six developed no symptoms and one slight only.

(b) Six rats were fed on excess fat carbohydrate free diet for 13

days; insulin caused no symptoms in any of these; after feeding for 13 days on the complete diet, four developed severe symptoms in 1 hr. 40 mins. to 2 hours, one had less severe symptoms and only one had none.

In addition to this series of experiments (on 128 rats) a second series was made with different insulin, the strength of which was possibly rather lower than that used in the first series. The dose of insulin and the conditions were the same as in the first series. The rats used were placed on the diet about six weeks after birth, their weight being about 40 grms. They were kept on this diet for a period varying from three to ten weeks before injection, the average time being under six weeks. The results of the two series are given in Table IV.

TABLE IV.

Diet	No. of rats injected	Dead	With symptoms	Percentage
Complete	118	24	59	70.3
Excess-fat	131	2	18	15.3

TABLE V.

Cod liver oil, excess-carbohydrate. No fat	19	—	14	73.6
Complete	22	—	14	63.6
Excess-fat, no carbohydrate	22	—	3	13.6

In a few experiments rats were fed on a diet containing no butter. The calorie value of the diet was maintained by an increased proportion of starch (see Table I). The necessary amount of vitamin A was supplied by cod liver oil—0.3 c.c. of which was given daily by pipette to each rat on the no-fat diet. The results are summarised in Table V.

*Experiments on mice.* Similar experiments were made with mice instead of rats. The mice were kept under suitable conditions in large wooden boxes and were fed on the diets for about a week before injection. The diets used for mice were the same as those used for the rats except that cod liver oil was added to each diet. The amount added in Series 4 (Table VI) was equivalent to over 5.0 mg. per rat and it was assumed that this would be sufficient for an equal weight of mouse. The mice

TABLE VI. Mice. Series 3.

Dose in chemical units per 20 grm. mouse	Excess-fat diet			Complete diet			No fat excess-carbohydrate		
	No injected	No. symptoms	Percentage	No injected	No. symptoms	Percentage	No injected	No. symptoms	Percentage
0.1	20	3	15	20	1	5	20	2	10
0.13	27	1	3.7	37	9	24.3	42	19	42.2
0.2	40	7	17.5	40	22	55	36	23	63.9

Series 4.

0.13	51	7	13.7	60	38	63.3	62	47	75.8
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in Series 3 (Table VI) received ten times this amount in their diet with the exception of mice on the complete diet from which the cod liver oil was unfortunately omitted. The mice ate almost all the food provided for them during the twenty-four hours. Table VI shows the results.

All these results seem to show clearly that a diet from which carbohydrate is completely absent and which contains in its place an increased amount of butter-fat, produces in rats and mice a decreased sensitivity to insulin. Whether the further deduction can be made, that the reduced sensitivity is proportional to the amount of butter-fat present is less certain. Though the difference in the percentage number of symptoms on the complete and excess carbohydrate no fat diets is probably a significant figure, this cannot be held to be definitely established. It is assumed that the important factor is the presence of increased amounts of fat and not the absence of carbohydrate.

A few rats were put on a carbohydrate-free diet in which the butter-fat was replaced by heated lard (21 grms). These rats were old stock rats weighing from 150 to 250 grms at the beginning of the experiment. They were injected with the usual dose of insulin a month later. Table VII gives the results compared with those shown by similar rats on the complete and excess butter fat diet. All the rats were injected at the same time. One rat only on the excess-butter-fat diet showed more than very slight symptoms and those developed by the rats fed on the diet containing the lard were almost equally slight.

TABLE VII

Diet	No. of rats injected	Dead	Other symptoms
Complete	10	2	8
Excess butter fat	10	none	3, 2 very slight
Excess (lard) fat	9	none	1, all slight

*Blood sugars.* The blood sugar was estimated in a small number of cases. Miss Boock kindly bled the rats for me by cardiac puncture. The average blood sugar of 10 normal rats was found to be 0.13 p.c. That of dieted rats one or two hours after injection is shown in the following table (Table VIII).

TABLE VIII Blood sugars of rats 1-2 hours after injection with insulin

Diet	No. of rats	With symptoms	Average blood sugar values	Lowest value	Highest value
Complete	11	10	0.096	0.075	0.12
Excess fat	13	4*	0.085	0.074	0.12

\* Only slight symptoms in each case.

There are various other factors which might influence the results.

*Condition of animals.* The rats on all diets appeared to be strong and healthy throughout the experimental period. The gain in weight of rats on the excess-fat diet was at least equal to that of rats on the complete diet. The gain in weight in four weeks of 71 rats on the complete diet was 104 p.c., that of 67 rats on the excess-fat diet was 135 p.c.

The mice on the excess-fat diet were in bad condition after two or three days and got progressively worse. Mice on the other two diets remained well and healthy (Table IX).

TABLE IX. Relative mortality of mice on diets for 6 days.

Diet	No. of mice	Deaths in 6 days
Carbohydrate only (No fat)	100	3
Complete	100	6
Excess-fat (No carbohydrate)	100	32
Excess-fat (No carbohydrate) Phosphate water	50	2

During the six days the 100 mice were on the excess-fat diet, there were thirty-two deaths. Eighteen of these deaths occurred overnight among seventy of these mice, which were being starved for injection. 50 of these 52 mice alive next day were given .013 clinical unit of insulin and only seven of the 50 showed any symptoms, whereas the corresponding figures, after the same dose of insulin, for complete and carbohydrate diet mice on the same day were 27 out of 39 injected and 31 out of 40 respectively. The mice on the excess-fat diet had no doubt developed a considerable degree of acidosis, and looked ill, with rough greasy coats and many were in a state of coma before death. A 5 p.c. solution of  $\text{Na}_2\text{HPO}_4$  in tap water had been substituted for the ordinary drinking water in the case of other fifty mice on the excess-fat diet with the result that the mortality before injection was only 4 p.c.; their response to insulin was exactly the same as that of the excess-fat mice which developed acidosis.

*Action of cod liver oil.* The different susceptibility to insulin of rats and mice fed on diets differing in their contents of butter-fat does not appear to be due to a deficiency of vitamin fat—soluble A in the diet containing the smaller amount of butter, as the addition of an amount of cod liver oil more than sufficient to supply an adequate amount of this vitamin has no influence on the results. Table X shows the results of insulin injection on dieted rats with and without cod liver oil, and they are confirmed by the experiments on mice already referred to.

TABLE X Rats fed on diets with and without cod liver oil

Diet	Plus 0.4 c c cod liver oil daily			Diet without cod liver oil		
	No injected	With symptoms	Percentage	No injected	With symptoms	Percentage
Complete	12	9	75	12	10	85
Carbohydrate free	26	11	42	23	11	47

*Importance of the thyroid* Dr Burn suggested that the resistance of rats on an excess-fat diet might be due to the action of fat on the thyroid gland. Burn<sup>(2)</sup> has shown that thyroidectomised rabbits are more than normally sensitive to insulin, but recover their normal resistance when fed with extracts of that gland. E and M. Mellanby found that puppies living in confinement show marked hyperplasia of the thyroid when fed on butter-fat, the weight of the gland increasing five-fold or even more. The thyroid glands of a number of these experimental rats were therefore dissected out and weighed as soon as possible after death. They were also examined histologically. The average weight of 18 rats on a complete diet was .0135 gm (standard deviation of mean = 0.000566), that of 21 rats on excess-fat diet was .0163 (standard deviation of mean = 0.00071), a difference just not large enough to be significant. No histological changes were obvious.

*Results* The results show that a diet from which carbohydrate is absent and which contains excess fat reduces the sensitivity to insulin of the animals tested to such an extent that a dose which would cause 70 p.c. of those on the complete diet to convulse causes symptoms in under 20 p.c. of the cases on the excess-fat diet, no explanation has been satisfactorily demonstrated. The variation in sensitivity is apparently not due to variations in the amount of vitamin present and it has not been proved that it is due to variations in the activity of the thyroid gland.

#### SUMMARY

1 Rats and mice were fed on a diet containing protein, salts and vitamins, from which carbohydrate was omitted, the necessary calorie value being maintained by an increased amount of fat. Such animals developed a high degree of resistance to insulin.

2 Certain experiments make it appear probable that an increased amount of carbohydrate and complete absence of fat in the diet render the animals still more sensitive to insulin than they would be on a complete diet, but this cannot be considered to be definitely proved.

3 The variations in sensitivity are not due to deficiencies in the amount of vitamin A present.

4. The evidence as to the importance of the thyroid gland is inconclusive.

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- (1) Halliburton and Drummond. *This Journ.* 51. p. 235. 1917.
- (2) Burn and Marks. *Ibid.* 39. 1924; *Proc. Physiol. Soc.* p. viii.
- (3) E. and M. Mellanby. *Ibid.* 55. 1921; *Proc. Physiol. Soc.* p. vii.

I am much indebted to Dr Trevan for help and advice in writing this paper.

THE SPREAD OF ACTIVITY IN THE TENUISSIMUS  
MUSCLE OF THE CAT AND IN OTHER COMPLEX  
MUSCLES. By E. D. ADRIAN.

*(From the Physiological Laboratory, Cambridge)*

THE muscle from which we derive most of our information about the conduction of the mechanical and electrical effects is the sartorius of the frog. The arrangement of long, parallel fibres in this muscle makes it easy to record the development of activity in successive segments and to deduce the behaviour of the single muscle fibre from that of the entire muscle. That the spread of activity follows the same general course in mammalian muscles was shown many years ago by Rollett<sup>(1)</sup> and by Bernstein and Steiner<sup>(2)</sup> who measured the rate of travel of the contraction wave and electric response in the curarised sterno mastoid. There remains, however, a good deal of uncertainty in our ideas of the spread of activity, particularly the electrical activity, in more complicated muscles, and Henriques and Lindhard<sup>(3)</sup> have rightly drawn attention to certain difficulties in the usual interpretation of the electric response. The present work was begun in the hope of finding a good preparation for the study of conduction in the muscle fibre, it has shown instead a preparation illustrating with great clearness the differences between the spread of activity in the fibre and that in the entire muscle.

The tenuissimus in the cat is a long and very slender muscle running from the caudal vertebrae to an insertion halfway down the leg. It varies from about 9-15 cm. in length and 2-4 mm. in width and, according to Porter and Hart<sup>(4)</sup>, a cross section contains approximately 1000 fibres. The muscle fibres are parallel and there are no tendinous inter-sections of any kind. The nerve arises from the sciatic as a slender twig which branches into two, just before entering the muscle, about a third of the way down. It will be convenient to speak of the part of the muscle distal to the entry of the nerve as the tibial portion and that proximal as the pelvic portion. Preparations of the muscle with the circulation intact have been used by Graham Brown<sup>(5)</sup> and by Porter and Hart<sup>(4)</sup> for investigating the all-or-nothing principle in reflex contractions, and for this the tenuissimus is admirably suited on account of the small number of nerve fibres in the branch supplying the pelvic portion. In the present

work the muscle was sometimes left *in situ* and merely exposed by removing the outer portion of the biceps in a spinal (decapitated) cat, but in many experiments it was removed from the body and set up in a moist chamber at 35° C. or in a bath of warm, oxygenated saline. In these conditions the muscle contracts on stimulation for upwards of two hours, and provided the stimulation is not repeated too often it shows no signs of any falling off in activity compared with a muscle with circulation intact. Indeed Riesser (6) has shown that much more bulky mammalian muscles will retain their activity in the same way in oxygenated saline.

#### A. Spread of Contraction.

The sciatic nerve is cut through high up in the thigh to prevent reflex effects and the muscle is stimulated by a break induction shock led in through platinum electrodes 3 mm. apart. If these are applied anywhere but at the extreme pelvic end, the stimulus usually produces a contraction which can be seen to involve most of the muscle (see Fig. 1 *a*). A stimulus at the pelvic end or in the region midway between

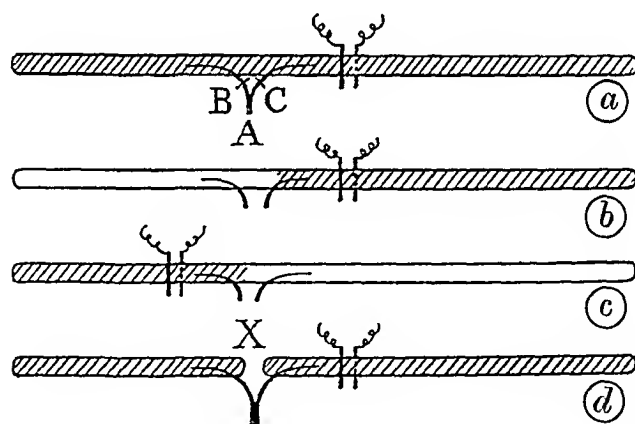


Fig. 1. Extent of contraction in tenuissimus muscle with various arrangements of electrodes, section of nerve branches, etc. The contracting part is shaded.

the points of entry of the two nerve branches may sometimes give a local contraction confined to a few centimetres only. If, however, the two branches of the nerve trunk are cut through (Fig. 1 *b* and *c*) a stimulus applied to the tibial two-thirds of the muscle gives a contraction confined to that portion only, and a stimulus to the pelvic third gives a contraction confined to that portion. If the ends of the muscle are fixed the contraction of the tibial portion produces an elastic extension of the pelvic and *vice versa*. Evidently then the spread of contraction from the tibial

to the pelvic portion is due to conduction through the two nerve branches and not to conduction through the muscle itself. This may be confirmed by cutting through the muscle at *X* (Fig. 1 *d*), leaving the nerve branches intact. In spite of this section both parts of the muscle contract when one is stimulated. Both nerve branches pass up and down in the substance of the muscle, so that one or other would be stimulated by a shock applied to the muscle directly, but to account for the activation of one branch when the other is stimulated we must suppose that the nerve fibres in the two branches are formed by the bifurcation of parent fibres in the common nerve trunk. That such a bifurcation does take place can be shown by counting the nerve fibres in the main trunk at *A* (Fig. 1) and in the two branches at *B* and *C*. The total number of fibres found at these cross-sections in four animals are given in Table I, and it will be seen that the number of fibres in the two branches added together is always much greater than the number in the main trunk.

TABLE I. Total number of medullated fibres in cross-section of nerve.

A. Main trunk	B. Pelvic branch	C. Tibial branch	Both branches
85	56	75	131
92	55	72	127
79	55	74	129
70	35	63	98
58	29	56	87

Thus the spread of activity throughout the muscle when one point is stimulated is due to the excitation of nerve fibres which branch in such a way that one nerve fibre of the main trunk may be connected with muscle fibres at either end of the muscle. The spread is in fact an axon reflex of the type shown by Kühne in the gracilis, though the present case is interesting in that there is no obvious break in the continuity of the muscle, and that the same parent nerve fibre may supply nerve endings in two regions distant as much as 10 cm. from one another.

*Effect of curare.* To eliminate the effects of nervous conduction 1-2 c.c. of a 1 p.c. solution of curare was injected into the spinal preparation. This was enough to abolish all movement when a motor nerve was stimulated. Single or tetanic shocks applied to the tenuissimus now produced a localised contraction confined to 2 or 3 cm. in the neighbourhood of the electrodes, the rest of the muscle becoming passively extended. The length of the muscle which participates in this localised contraction was determined by slinging it horizontally by a number of silk threads attached to it at intervals of 1 cm. and carried vertically upwards to a support about 50 cm. above. The threads were illuminated by an arc lamp so that their shadows fell on the slit of a moving plate

camera. If any part of the muscle contracts, the threads attached to that part move nearer to one another and their movement can be recorded photographically. The method is in fact an adaptation of the polymyograph used by Lewis, Feil and Stroud (7) for following the spread of contraction in heart muscle. Records with the curarised muscle agree in showing a contraction localised to about 1.5 cm. on either side of the cathode, and the same localised effect is produced when the uncurarised muscle is stimulated at the extreme pelvic end where very few nerve fibres are present, or in the nerve-free region between the point of entry of the two branches. The extent of the spread is not appreciably affected by changes in the intensity of the stimulating current up to five or six times the threshold strength, or by using the make or break of a constant current instead of an induction shock.

Granting, then, that the limited spread of the contraction is a normal event on direct stimulation of the muscle, there are two possible explanations which suggest themselves. The first is that the fibres of the muscle conduct with a decrement, so that the disturbance dies out after travelling for a short distance; the second is that the muscle fibres are in reality only a few centimetres long although they appear to be continuous throughout the length of the muscle when examined without special preparation. There is no doubt that the second explanation is correct. If the tenuissimus is extended by a small glass weight and macerated for 24 hours or more in 20 p.c. nitric acid and the fibres are then separated by shaking, it will be seen that they are rarely longer than 2 cm. and that they taper gradually and end in a long, pointed process. Table II gives the lengths of 21 unbroken fibres from one muscle 12 cm. long. It is possible that there are some longer fibres which break up during the process of isolation, but I have not succeeded in tracing one longer than 27 mm.

TABLE II. Length of individual fibres in cat's tenuissimus muscle.

15 mm.	16 mm.	17.25 mm.	14.5 mm.
16	17	18	19
18.5	19.5	16.5	16.5
13.8	22.5	25.5	22.5
14	16.5	13	15
17			

Average 17.3 mm. Extremes 13 mm., 25.5 mm.

Thus the tenuissimus is made up, not of long fibres continuous from one end to the other, but of fibres about 2 cm. long arranged in series with their pointed ends dovetailed between other fibres and attached to the endomysium. The arrangement is shown diagrammatically in



Fig. 3, and it is one which is not uncommon in any long muscle(s). According to Mayeda(9) even the sartorius of the frog contains some fibres only a few mm. in length and the average length of the fibres in a muscle 26 mm. long is only 20 mm. It is true that the possibility of decremental conduction cannot be set aside, for the contraction involves only 2-3 cm. of the muscle whereas the stimulus ought to affect all fibres coming under the electrode region and these should stretch out 1.5-2 cm. on either side of it. Probably these fibres produce no visible shortening because they are mingled with inactive fibres not affected by the stimulus. There is some evidence of decremental conduction in the frog's sartorius after prolonged tetanisation (Lucas(10)), or two months after denervation (Adrian and Owen(11)), but Lucas and Bethe and Happel(12) agree in finding no evidence of it in the normal muscle and in the present case it is unlikely that such a state would last unchanged for an hour or more.

### B. *Electric response.*

The electric responses given with different leads are a good example of the effects obtained in a muscle whose fibres are not long enough to stretch from one end to the other. In the uncrurised muscle, if the stimulus is applied so as to excite one or other of the nerve branches and produce a widespread contraction, an electric response is obtained if one of the leads is anywhere on the contracting part of the muscle, but not if both are on the part which is passively extended. The response may show two peculiarities: it may be polyphasic instead of diphasic, and it does not necessarily become monophasic when the muscle is injured under one electrode.

*Effect of nerve endings.* Before these peculiarities are discussed, it is necessary to decide a point concerning the general interpretation of the electric response on which some doubt has arisen. What may be called the classical theory assumes that the action current of a muscle is due, like that of a nerve, to a disturbance which travels down the fibre producing a momentary change of potential in each section through which it passes. Henriques and Lindhard(3) have recently put forward an alternative view that the electric response led off from a muscle is due to a current generated at the nerve ending, and that the muscle fibre itself does not contribute to the response. This view was criticised by Adrian and Owen(11), who carried out what seemed to be the crucial test of finding whether a denervated muscle would still yield an electric response. They found that it would do so and that the response differed in no important respect from that of a normal muscle. In a later paper,

however, Henriques and Lindhard<sup>(13)</sup> have suggested that the result was vitiated by current spread from the stimulating electrodes and have reaffirmed their view of the absence of response from the muscle fibre.

As the point is vital to the present argument it seemed worth while to repeat Adrian and Owen's experiments. These had been carried out with the capillary electrometer and with this instrument the inertia factor is so small that it is easy to distinguish the sharp initial excursion due to the stimulating current from the slower action current which follows it. The time relations of the responses found by Adrian and Owen were those of an action current and not those of a stimulus escape; moreover the response became monophasic when the muscles were damaged under one electrode and this would be, to say the least, an unusual occurrence with a pure artefact. The experiment has been repeated with the string galvanometer instead of the capillary electrometer, since the records of the former are more generally familiar. The muscle used was the frog's sartorius denervated from five to eight weeks previously by section of the sciatic above the branch to the sartorius, and the denervation was checked by methylene blue staining of sample muscles. To distinguish between the spread of the stimulating current and a true response of the muscle, make and break shocks were used alternately so as to reverse the direction of the artefact, a coreless coil was used so that its duration would be very short, and at the end of the experiment records were made after the muscle had been killed *in situ* by pouring hot Ringer's fluid on it, the electrodes, etc. being disturbed as little as possible.

Six muscles were tested and all gave an electric response, diphasic when the muscle was uninjured, monophasic when it was injured at one end and clearly distinguishable from the escape of the stimulating current. With a stimulus not more than three or four times the threshold strength the artefact was usually so small as to be scarcely visible. Typical records are shown in Fig. 2 and they seem to leave no doubt that a denervated sartorius continues to give an electric response of the usual type. We may conclude therefore that the presence of an intact nerve ending is not necessary for the development of the electric response in a muscle fibre, and that, from this evidence at least, there are no grounds for rejecting the classical explanation.

*Monophasic responses.* With a simple tissue—a nerve or a long muscle fibre—if the region under one electrode, or anywhere between the electrodes is damaged, the response is always monophasic whatever the distance between the electrodes may be. With the tenuissimus the

usual injury current is produced, but the response is not strictly monophasic unless the proximal electrode is less than about 1 cm. from the damaged area. If the distance between the electrodes is increased to

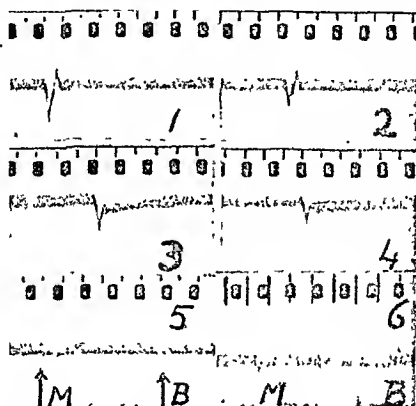


Fig. 2. Elective response of frog's sartorius 8 weeks after denervation.

1. Muscle uninjured. Diphasic response to make induction shock (3.5 times threshold strength).
2. Muscle uninjured. Diphasic response to break induction shock (3.5 times threshold strength).
3. Tibial end damaged. Monophasic response to make shock (3.5 times threshold strength).
4. Tibial end damaged. Monophasic response to break shock (3.5 times threshold strength).
5. Muscle killed. Make and break shocks 3.5 times threshold strength; scarcely any current escape.
6. Muscle killed. Make and break shocks, 36 times threshold strength.

String galvanometer with  $3.5 \mu$  silvered glass string, resistance 3750 ohms. Magnification 400. Tension, 1 mv. through string gives 3 mm. deflection on film. Time marker gives  $\frac{1}{3}$  second.

3 cm. or more, it would be extremely difficult from an inspection of the record to tell whether the muscle had been damaged or not (Fig. 5).

This result has both a theoretical and a practical interest. Theoretically it agrees very well with what is known as the membrane hypothesis, the view that the action and injury currents are due to a change in permeability at the active surface which allows a free inter-

change of the ions normally present inside and outside the fibre, the change being reversible in the case of activity and irreversible after injury. It is assumed that there is normally a difference of potential between the inside and the outside of the fibre determined by the nature of the surface membrane and the unequal distribution of different ions, and that the injured or active fibre behaves like a concentration cell, the current flowing through the circuit which connects the intact surface with the active or injured region, since the electrode on this region may be regarded as leading off from the interior of the fibre. When one end of the fibre is injured and electrodes are placed on this end and on the intact surface, stimulation will produce the momentary cessation of the injury current which constitutes the "negative variation." The passage of the active region past the proximal electrode and towards the injured area will allow the injury current to reassert itself, but will not make it any greater than before, since there is already a free passage of ions through the injured cell boundary and the approach of the active region will not make it more free. Thus the change will be monophasic.



0 5 1 1.5 2 cm.

Fig. 3.

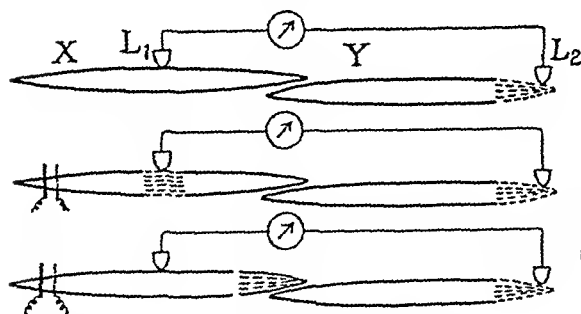


Fig. 4.

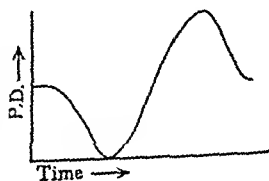


Fig. 3. Arrangement of muscle fibres in tenuissimus.

Fig. 4. Production of diphasic response with two fibres, X and Y, in series. X alone is stimulated; Y has been injured at one end. Dotted areas represent active or injured surface.

The conditions are entirely different if the system is made up of two fibres in line and one of them is damaged (Fig. 4). To simplify matters,

assume that  $Y$  remains inactive whilst  $X$  contracts. There will be initially an injury current owing to the fact that  $L_2$  is in connection with the interior of the fibre  $Y$ , whilst  $L_1$ , through  $X$  and the surrounding fluid, is in connection with its intact surface. When the part of  $X$  under  $L_1$  becomes active the injury current will disappear, for conditions under  $L_1$  and  $L_2$  will then be identical, but eventually the active region will pass to the distal end of  $X$  and we shall then have the active surface of  $X$  in contact with the intact surface of  $Y$ . If each fibre behaves as a concentration cell the combination of the two in series should give twice the E.M.F. of one alone. So, when  $X$  is stimulated, the injury current will first disappear and will then rise to twice its normal value, giving a diphasic instead of a monophasic response. The conditions are easily reproduced by placing two sartorius muscles from the frog in series and damaging one at the tibial end. When the first muscle is stimulated the usual diphasic response is always given. In the case of the tenuissimus, if the nerve is stimulated the fibre  $Y$  will be active as well as  $X$ .  $Y$  alone would give a monophasic response and the effect of this will be to make the first phase of the combined response greater than the second, but it will not prevent the appearance of the second phase.

If we can safely regard the action current and injury current as due to the same cause, these results are a logical outcome of the fundamental observation of Brunings<sup>(11)</sup> that two sartorius muscles damaged at their tibial ends and placed in series give an injury current with an E.M.F. twice that given by one alone. This is reasonable enough if the damaged fibres behave like concentration cells with a potential difference at the intact surface, but it is very difficult to reconcile with any hypothesis which leaves the structure of the fibre out of account and makes the current depend on the sudden production of an excess of ions at the active region and the setting up of diffusion or concentration potentials at the electrodes. Unless there is something in the structure of the fibre which directs ions of opposite sign along different channels (i.e. an intact surface permeable only to ions of one sign and an active region permeable to both) there is no reason why the E.M.F. of active or injured fibres in series should be any greater than that of a single fibre.

Practically these results emphasise the fact that it is impossible to record a response which is truly monophasic—i.e. which is determined entirely by the change under one electrode—unless both electrodes are confined to the same group of muscle fibres. To secure this, the maximum distance between the proximal lead (that nearest the point of stimulation) and the damaged region must be shorter than the length of a single

response at any point in the fibre (*e.g.* the monophasic response of the frog's sartorius) is increased to about three times its former value by a fall of  $10^{\circ}$  C. (16), but the intervals between the arrival of disturbances in different fibres will depend on the rate of conduction and this is only increased 1.8 times by a  $10^{\circ}$  fall (17). Cooling will therefore produce a much greater overlapping between successive phases and the smaller oscillations may be smoothed out altogether. Whether this explanation will cover all of Judin's results is a point which cannot be decided in the absence of further experimental details, but its possibility must be considered in any attempt to account for the polyphasic response.

A necessary consequence of the present explanation is that the polyphasic response should only appear when the distance between the electrodes is great enough to include fibres whose active regions will not all coincide. In the *tenuissimus* there is often a second upward deviation although the distance between the electrodes is reduced to less than 1 cm., but the arrangement of the fibres is such that many would be ending at different points in the interpolar field. The effect of changing the distance between the electrodes is better seen in a muscle like the soleus where the fibres are arranged obliquely and end in a tendinous expansion.

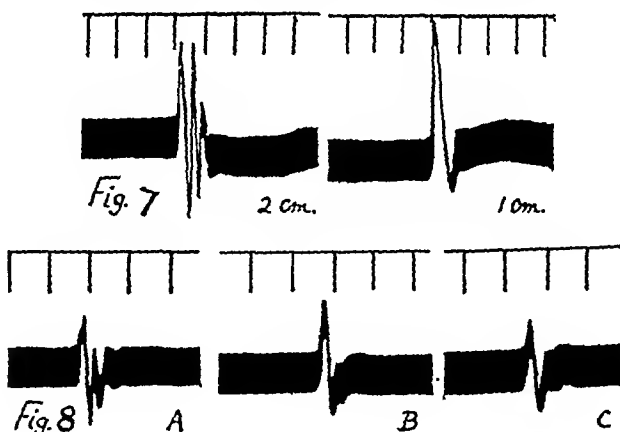


Fig. 7. Response of cat's soleus stimulated by a break shock to the nerve. One electrode on distal end of muscle (injured by burning), the other 2 cm. or 1 cm. higher up. Polyphasic response with 2 cm. between electrodes.

Fig. 8. Response of cat's gastrocnemius stimulated by break shock to the nerve:  
 A. Small electrodes 3 mm. diam., 2 cm. apart.  
 B. Same electrodes 1 cm. apart.  
 C. Large electrodes 1.5 cm. diam., 2 cm. apart (3.5 cm. between centres).

Fig. 7 shows two responses from the soleus of a spinal cat stimulated by a single break shock to the popliteal nerve. The muscle had been damaged

under the lower electrode, but neither response is monophasic. With a separation of 2 cm. between the electrodes there are no less than four upward excursions of the string. With a 1 cm. separation these are reduced to a single excursion. Similar effects are given by the gastrocnemius and the quadriceps.

*Effect of electrode area in complex muscles.* If these complex responses are caused by a lack of synchronisation in the disturbances entering the interpolar field, they should be modified by any factor which changes this field, i.e. they should depend on the area of the electrodes as well as on the distance between them. In the tenuissimus the muscle is so narrow that a restriction or expansion of electrode area is not likely to have much effect, but in a more bulky muscle like the cat's gastrocnemius an increase in the size of the electrodes has much the same effect as a reduction in the distance between them. This may be seen from Fig 8 which compares the response of the gastrocnemius (a) with small non-polarisable electrodes 3 mm. in diameter and 2 cm. apart, (b) with the same electrodes 1 cm. apart, and (c) with large pad electrodes 1.5 cm. in diameter and 2 cm. apart (3.5 cm. between centres). There is little difference between (b) and (c), but (a) shows a much greater prominence of the later phases.

The simplest way of regarding these effects is to suppose that with electrodes of large area the changes of potential in the fibres near the electrodes become averaged out and the response is determined mainly by the fibre groups which lie midway between the two poles. With small electrodes the interpolar field is much more sharply defined and a few fibres responding out of phase with the rest will have a much greater effect on the total response. In fact with very small electrodes the response is mainly determined by the fibres which lie in the straight line between them, with large electrodes the response is an average for the whole muscle. The point has been recognised in the use of needle electrodes for recording the electromyogram, and Bass and Trendelenburg (18) have shown that with needles embedded in a large muscle the action current in fibres a few cm. away has no appreciable effect on the galvanometer. With large pads, on the other hand, the response may be due to almost any muscle in the limb.

*Red and white fibres.* One other possible cause of complex responses is the occurrence of fibres the electric responses of which have widely different time relations, as have the fibres of the red and white muscles of the rabbit. Erlanger and Gasser (19) have shown how polyphasic action currents may be given by a nerve trunk in which there are groups

of fibres conducting at different rates and the same thing might well occur in a mixed muscle. The cat's tenuissimus shows a mixture of clear and opaque fibres in cross-sections, but it is impossible to say how far they contribute to the complex response, as the other cause—the short length of the fibres and their arrangement in series—cannot be completely eliminated.

One practical outcome is that if we wish to determine the frequency at which the muscle is activated in given circumstances we must arrange the electrodes so as to minimise the chances of recording a polyphasic response from a single activation of the muscle. This can be done by making the effective interpolar field so small that most of the action current will come from a single group of muscle fibres, or so large that the responses out of phase will neutralise one another's effects, leaving the average response from a large number of fibres. The former method is clearly preferable, for the average response from a large muscle will have little meaning unless we can assume that each group of fibres is activated at much the same frequency and more or less simultaneously.

Even with an extremely restricted interpolar field the galvanometer record from a complex muscle cannot be trusted to give a true picture of the response in a single fibre. Forbes, Ray and Hopkins<sup>(20)</sup> have shown how a change of initial tension may modify the action current of the gastrocnemius by altering the relations of adjacent fibres, and it is doubtful if we can ever draw definite conclusions as to the form and amplitude of the action current in such muscles unless these are checked by reference to a response which is truly monophasic. This can only be recorded by using a muscle with parallel fibres injured at one end and long enough to fulfil the condition that no active, uninjured fibres may extend into the interpolar field.

#### SUMMARY.

The tenuissimus muscle in the cat is over 10 cm. long; it is a few millimetres wide and it is made up of parallel fibres each about 2 cm. in length and held together by the fibrous endomysium. This arrangement makes it a suitable muscle for illustrating the response of a complex muscle whose fibres do not stretch from end to end. Thus a stimulus to the curarised muscle produces a contraction limited to 1.5 cm. on either side of the stimulated point, and an electric response is only obtained if one electrode is on a region which contracts. In the uncurarised muscle the stimulus takes effect on the intramuscular nerve trunk and a larger area contracts. The electric response may be



polyphasic, the separation between the phases does not vary in a regular way with the distance between the electrodes and the response does not become monophasic when the muscle is damaged under one electrode unless the distance between the electrodes is reduced to 1 cm or less. These features of the response are shown to agree with the prevailing views as to the nature of the action and injury currents, and the conditions determining polyphasic and monophasic responses are examined in some other complex muscles.

The expenses of this work were defrayed in part by a grant from the Government Grants Committee of the Royal Society.

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# THE EFFECT OF THE CIRCULATION ON THE ELECTRICAL RESISTANCE OF THE SKIN.

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King's College, London.)*

OF recent years, especially among psychologists, considerable attention has been drawn to the fact first noticed by Féré, that under conditions of emotion the electrical resistance of the skin of man may undergo considerable reduction, and this has given rise to a large number of observations in man on what is called the psycho-galvanic reflex. The efferent nervous impulses of the reflex are generally held to pass to the skin by way of the sympathetic nerve fibres; *i.e.* either by vascular or sweat fibres. The vascularity of the skin can be modified in a number of ways and it seemed desirable to determine how far the skin resistance varied with its vascularity.

Only very few investigators appear to have succeeded, for reasons indicated below, in bringing about any change in resistance in anæsthetised animals, and those have confined themselves to a study of the nature of the sensory stimuli which produce the fall or to the study of the relation of the fall of resistance to the possible action of the sweat glands. Attention to the latter has been rather emphasised by the fact that some observers have found that the fall of resistance was abolished by atropine (Sehlf and Schubert, Fauville). It appears however to have been completely overlooked that the effect of atropine is not confined to its action on glands and that it has a profound effect on blood vessels. Similarly it has been considered that the persistence of the fall of resistance after temporary occlusion of the blood supply to the heart necessarily indicated that it was not due to vascular changes. As we now know that the capillaries are largely independent of the arteries it will be clear that such evidence can no longer be taken as conclusive.

Advantage was taken of the fact that the vasomotor system of animals under chloralose is appreciably more sensitive than if ether is used, especially if the experiment is prolonged. The experiments were made on cats. Ordinary zinc wash-leather electrodes were lightly bandaged on to the skin, the fur being clipped off if necessary with scissors and the whole soaked in saline. The electrodes were connected with a Wheatstone bridge and a galvanometer in the ordinary way used for observing resistances. In different animals the resistance varied from 30,000 to 60,000 ohms, but subsequently it was found could be changed

as desired by the experimental procedures. Changes in resistance varied up to 2000 ohms. That these changes were really due to the skin could readily be demonstrated by removing it when it was found that the resistance of the circuit was less than 2000 ohms and the changes in this resistance were of such a small order as to be negligible. The results obtained were extremely definite provided fresh animals not suffering from shock were used. It was found from time to time that the reactions given before the preliminary ether anaesthesia had worn off were absent or were appreciably less than those given later under the chloralose. This is important and no doubt accounts for the completely negative results which have been recorded by other workers.

*Direct vaso constriction* This was brought about by the intravenous injection of adrenaline (1/50 mg), which was found to bring about a marked fall in the resistance of the skin of the limbs and the pads of the feet. The fall of resistance corresponded with the usual rise of blood-pressure when the drug is injected. The return of the skin resistance to its previous height synchronised with the return of the blood-pressure. The change in the skin resistance like the rise of blood pressure was best seen when the vagi were cut.

*Indirect vaso-constriction* It is well known that in hemorrhage there is constriction of the skin vessels as a result of increased action of the vasomotor centre. This is indicated by the pallor. When the animal was bled from the carotid artery there occurred a marked fall of skin resistance.

Of interest in this respect was the effect of small doses of histamine (1/10 mg), which have been shown by Dale and Richards to bring about capillary dilatation. The recovery from such small doses is extremely rapid and this has been shown by McDowall and Worsnop to be due to reflex arterial constriction through the operation of the vago-pressor reflex, and they have drawn attention to the similarity in the effect of the circulation of the arteries of such small doses to the effect of small hemorrhages. The similarity between large hemorrhages and large doses of histamine from which the blood pressure does not recover was pointed out by Dale and Laird. It was found that on the injection of a small dose of histamine there was a decided fall in the skin resistance which persisted after recovery of the blood pressure. In this we have further evidence of the similarity referred to above between the action of hemorrhage and histamine. It is of interest to note in this respect that clinically in conditions of shock due to toxin absorption from wounds, there is a marked pallor of the skin and a sensation of coldness to the touch.

A similar fall in skin resistance was brought about by the action of cold which we know brings about a constriction of the skin vessels in order to prevent heat loss. Cold was applied to the limb above the electrodes and to the carotid artery; and cold fluid was injected into the cephalic end of the carotid all with the same result.

*Vaso-dilatation.* This was brought about mechanically by compressing the inferior vena cava, when the electrodes were applied to the lower limbs. The procedure brought about a momentary fall of resistance but this was after half to one minute succeeded by a marked rise in skin resistance which persisted. This we may presume was brought about by the congestion of the part.

Dilatation of blood vessels was also brought about by the administration of amyl nitrite, which also caused a marked rise in the skin resistance.

Irritation of the part by plucking out the hairs previous to the application of the electrodes brought about a steady rise in skin resistance. When the electrodes were removed it was observed that the skin, previously pale, had become reddened as a result of the local dilatation of vessels.

A rise of resistance was also obtained by the injection of hot saline into the blood stream but the results, although definite, were not very marked.

It is then clearly evident that a vaso-constriction is associated with a fall in skin resistance and vaso-dilatation with a rise in skin resistance. A continuation of these experiments gave further results which could readily be interpreted from what has been said above.

*Asphyxia.* This was almost identical with the effect of clamping the inferior vena cava. There was a preliminary fall of resistance apparently due to the immediately stimulating effect of applying the clamp to the trachea, but thereafter there was a rise of resistance which continued to rise rapidly till the death of the animal. The procedure can readily be shown to result in an enormous raising of the pressure in the veins which we may infer brings about local dilatation of blood vessels in the region of the electrodes. No doubt the effect of  $\text{CO}_2$  also plays a part.

*Acetyl-choline.* If this drug is given in small doses insufficient to affect the heart its intravenous injection is associated with a rise in skin resistance. With larger doses, however, which brought about marked cardiac inhibitions there resulted a fall in skin resistance. Here we may understand that in the case of small doses there was the well-known vaso-dilator action of the drug, but with the larger doses when the

cardiac output was diminished as in hæmorrhage the compensatory vaso constriction counteracted the vaso dilatation and brought about the fall in the skin resistance. This explanation is supported by the fact that a similar fall of resistance occurs if the output of the heart is reduced by impeding its action mechanically with the fingers.

*Pilocarpine* The effect of this drug was specially interesting in view of its action on the sweat glands and in view of the controversy which had taken place in regard to the possibility of the secretion of sweat being responsible for changes in the skin resistance of man. Two distinct stages were usually seen. In the first there was a short but distinct fall of skin resistance, in the second a distinct and prolonged rise. The fall accompanied the evanescent pallor stage and the rise the flushed stage during which secretion takes place. The changes in skin resistance were obtained on the skin of the limbs which does not sweat. They were then clearly independent of sweating. This evidence, together with that given above, especially that in relation to cold, indicates clearly that sweating *per se* is not responsible for the fall in resistance. These results support those of Waller, who found that atropine given subcutaneously in sufficient doses to produce its well-known inhibition of the sweat glands did not interfere with the changes of skin resistance which occur in certain emotional states in man.

*Atropine* As indicated in the first paragraph the result of the injection of this drug is of special significance. The effect of the injection (1 to 2 mgm.) was clearly to bring about a steady rise in resistance of 10 or 15 minutes without recovery. This confirms the work of Markbeiter. In the early stages of such doses or if smaller doses were administered, it was possible to interrupt the rise by procedures such as the injection of adrenaline or sensory stimulation which we have seen above bring about a fall of resistance, although even at this stage it might be evident from the dilatation of the pupil and the acceleration of the heart that the parasympathetic had been paralysed (confirmation of Waller). These results are readily explained by the vaso dilator action of atropine. This action is often overlooked, though it is referred to in most of the pharmacological text-books and is well known in man.

When injected, the vaso dilator effect of the drug tends in the first instance to be masked by the accelerator effect on the heart which brings about a rise in blood-pressure. If, however, the vagi have been cut and the heart is already freed from vagus tone, or if the animal is suffering from shock, the same dose of atropine will be found to bring about a

profound fall of arterial pressure, which, indeed, may result in the death of the animal.

*Sensory stimulation.* The common stimulus adopted was pinching the skin of the fore limb with a pair of pliers. The stimulus resulted in a marked fall of skin resistance in the limbs or the pads of the feet. Repetition of the same stimulus did not always, however, bring about the repetition of the result which was normally of the same order as that obtained with a moderate dose of adrenaline (1/5 mg.). This change in reaction quite clearly did not depend on the higher centres as it was brought about in decerebrate cats.

*Remarks.* From what has been said it seems reasonable to believe that the fall of skin resistance which occurs on sensory stimulation is due to constriction of the skin vessels. The sensory stimulation is, as is well known, accompanied by a rise of blood-pressure as would be expected from sympathetic stimulation. That the sympathetic is stimulated is seen by evidence elsewhere than from the circulation, *e.g.* the dilatation of the pupil, which occurs at the same time. We have seen the similarity of result to the effect of adrenaline which is known to constrict the skin vessels. Further, under conditions, *e.g.* hæmorrhage, in which adrenaline no longer brings about a fall, there is no response to sensory stimulation although at the beginning of the experiment this could be readily obtained. The blood vessels may be presumed to be already constricted as the result of the effect of the hæmorrhage and cannot be made to constrict further by sensory stimulation. This presumption is supported by the fact that often in such circumstances the vessels may be dilated and the skin resistance made to rise by the administration of a powerful vasodilator, such as acetyl-choline and especially by asphyxia or clamping the vein.

This conception is also supported by Müller's results. He found in monkeys that section of the nerves supplying the heart only very temporarily reduced the fall of resistance obtained on sensory stimulation, although this was abolished by the local injection of 5 p.c. novocaine and adrenaline. It may be considered that the nerves concerned lie along the blood vessels, which, as we know, recover their tone fairly rapidly in spite of nerve section.

Thus procedures which are known to bring about the constriction of vessels cause a fall in the electrical resistance of the skin and a dilatation of vessels is associated with a rise in the resistance. All other procedures which affect the skin resistance of the chloralosed animal can readily be explained as being due to changes in the vascularity of the part. The

utility of such a relation is evident in the prevention of loss of blood and the throwing of a maximum amount of blood into the circulation. This will, of course, be prior to the requirements of skin dilatation for the purpose of increasing the heat loss once the exercise is begun. We have seen that the higher cerebral centres are not required and that the reaction is apparently of a reflex nature.

The reflex, however, does not appear to be limited strictly to the skin. A similar diminution is found to occur in muscles although in the latter instance the circuit has so little resistance that the changes which occur are very small compared with those of the skin.

Quite apart from the results obtained, it will be evident that we have in the investigation of the resistance of the skin, a method by which the circulation through it may be studied with accuracy, and it will be obvious that this may be of considerable value in the investigation of the location of the action of drugs especially in relation to the distribution of vasoconstriction and vaso dilator effects. It may also prove useful in relation to the study of sweat secretion.

#### SUMMARY

It is shown that in the cat a fall of the electrical resistance of the skin is associated with the constriction of vessels and that a rise is associated with a dilatation.

Reasons are put forward why the fall of skin resistance which occurs on sensory stimulation may be considered to be due to similar vasoconstriction.

The resistance of the skin may apparently be used as an indication of its vascularity.

In carrying out these experiments we are indebted for the assistance of Dr H. M. Wells and Dr R. C. McCarthy.

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In the above paper all reference to similar experiments in man have been omitted in view of the very voluminous and controversial nature of the literature and the fact that in man it is not yet clear that the changes in skin resistance may not be affected by other factors.

# A METHOD FOR THE PERFUSION OF THE KIDNEY WITH DEOXYGENATED BLOOD OF KNOWN OXYGEN TENSION.

BY E. W. H. CRUICKSHANK AND D. ORAHOVATS<sup>1</sup>.

*(From the Physiological Laboratory, Cambridge.)*

THE method to be described here is the result of experiments designed to supply blood of a definite percentage oxygen saturation to the kidney, or other organs of the body, while the rest of the body is supplied with blood having a normal oxygen content. In previous experiments on the effect of asphyxia on an organ such as the kidney, either the whole animal has been asphyxiated or the oxygen supply has been temporarily suspended by occlusion of the artery. In such conditions it is conjectural, how far the results obtained are due to simple anoxæmia of the organ and how far they are due to other conditions. It is important in perfusing an organ with defibrinated blood that there should be no cessation of its circulation. The difficulty which presented itself was how to take arterial blood, deoxygenate it and pass it to the kidneys. It will be seen that the idea which we wished to put into practice was the reverse of that of Richards and Drinker(1), who devised a very efficient apparatus for oxygenating blood; but if the dictum of Professor Starling be true, that it is impossible to construct an aerating apparatus for blood which will approximate to the efficiency of the lungs, much more is it true when one is faced with the problem of deoxygenating and equilibrating it with gases the tensions of which are totally different from those obtaining under normal physiological conditions. In order to secure efficient deoxygenation we considered, amongst other ways, the rapid evacuation of the gases of the blood and equilibrating the blood with a gas mixture in a large surface tonometer. The large tonometer and the large quantity of blood required for this made it too cumbersome to be practical. Mr Barcroft repeated to us a suggestion made to him by the late Professor Brodie, namely to pass the arterial blood of the experimental animal through the lungs of other animals breathing the required gas mixtures. It is on this idea that our method is based.

<sup>1</sup> Fellow of the Rockefeller Foundation.



### *Method*

Four cats were used for each experiment, one for the kidney, one for the artificially inflated lung preparation, and two for the additional supply of blood. It is essential that the whole system be kept filled with blood to the exclusion of air, no experiment should be commenced without at least 150 c.c. of blood in the reservoir. The cat which supplies the kidney is given an injection of urethane (1 c.c. of 25 p.c. per kilo), then the two additional cats are anaesthetised with c.c. mixture and bled, the blood being gently defibrinated and filtered through two layers of muslin. The urethamised cat is now eviscerated after the manner described by Cruickshank and Takeuchi(2), cannulae are placed in the right carotid and left external jugular vein, the first for a record of the blood pressure of the experimental animal, the second for the introduction of 150 mgms. of hirudin. If a good preparation is to be secured it is essential that the hirudin be injected slowly. If hirudin is not obtainable the animal must be defibrinated. We have had recourse to this procedure on three occasions because of the difficulty of obtaining hirudin. The cannula in the vein also serves, if necessary for the addition of blood to the animal, when there is any progressive fall in blood-pressure. The superior mesenteric artery is exposed and a cannula placed in it, the aorta is dissected out at a point just below the origin of the superior mesenteric artery and a double ligature placed round it and left loose. The aorta is exposed in this situation and not immediately above the renal arteries in order to prevent damage being done to the nerve supply to the kidney. It is rather difficult in the cat to dissect out the aorta below the suprarenals without some injury being inflicted upon the adjacent sympathetic ganglia. To restrict the blood supply to the lower part of the body the aorta is now tied just above the iliac bifurcation. The lumbar and ovarian veins are tied and cannulae placed in the aorta and inferior vena cava below the level of the kidneys. A piece of gauze moistened with warm saline is placed in the abdomen and the abdomen is partly closed with artery forceps.

*The preparation of the artificial lung.* The cat to be used is anaesthetised and bled, the blood being defibrinated, filtered and added to that already obtained. The chest is opened on the right side of the middle line so that the mediastinum on the left side remains intact, and thus the left lung is wholly covered and protected. By this means the onset of oedema is delayed considerably in the left lung. Given good inflation we believe that one lung so protected will afford ample means for com-

plete deoxygenation of the blood passing through it. As one must drain the pulmonary veins, which branch close to the left auricle, a cannula of the type shown in Fig. 1 must be used in order that no pressure be exerted on these veins. In order to proceed quickly with the insertion of the cannula the inferior vena cava is cut between double ligatures. The heart is raised by its apex and by means of an aneurism needle, a ligature is passed under the pulmonary veins, and brought forward between the aorta and left auricle, and under the arch of the pulmonary artery; this ensures an efficient tying of the cannula into the left auricle.

A ligature is now placed round the pulmonary artery, and wide mouthed cannulae are now inserted, tied into the left auricle and pulmonary artery, and supported together in a vertical position by means of a clamp. The lungs are washed out by means of a syringe, with warm saline or gum-Ringer solution; this must be done gently as any undue pressure will rupture the lung capillaries and thus spoil the preparation.

*Description of the system (Fig. 1).* The venous blood returning from

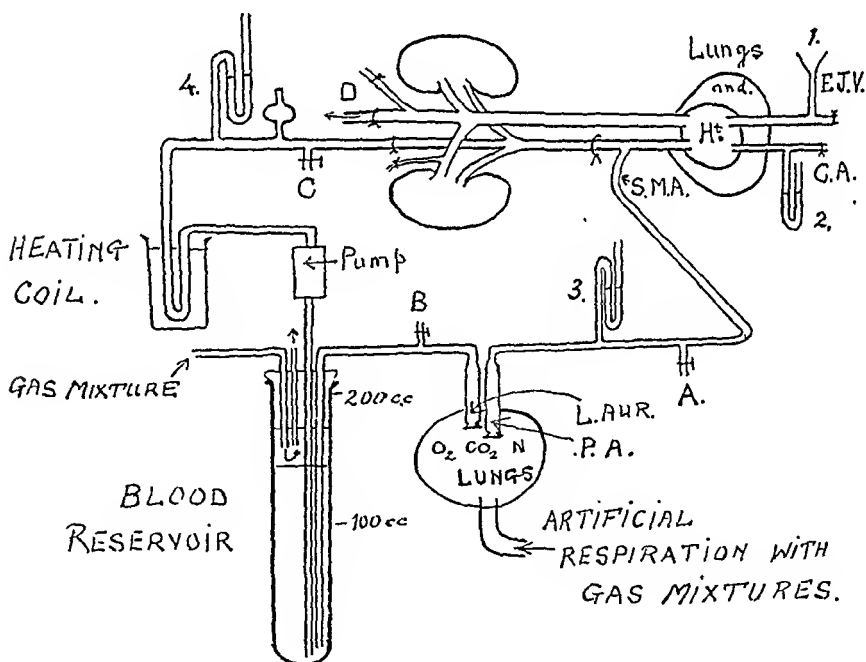


Fig. 1. Diagrammatic representation of the system for the supply of deoxygenated blood to the kidney.

A. Arterial blood samples; B. Deoxygenated blood samples; C. Deoxygenated blood entering kidney; D. Venous blood from the kidney.

1. Blood reserve; 2. Arterial blood-pressure; 3. Lung perfusion pressure; 4. Kidney perfusion pressure.

the kidneys is oxygenated in the lungs of the animal and is pumped by the heart to the head, body and fore limbs, part of the blood is allowed to pass from the superior mesenteric artery through an adjustable resistance to the lung of the second animal. The amount of blood passing by this route can be adjusted so that the level of blood in the reservoir is maintained constant, while the output by the pump is regulated to give a normal flow through the kidneys. The pressure under which the artificially inflated lungs are perfused should not, for the cat, exceed 20 mm Hg (3), otherwise the pulmonary artery will become distended and the lungs oedematous. A T tube is placed between the superior mesenteric artery and the lungs in order to take samples of oxygenated blood. The blood issuing from the lung of the second animal is allowed to flow into the reservoir, a T tube also being placed between the lungs and reservoir for the collection of samples of de-oxygenated blood. The reservoir is a large test tube ( $35 \times 250$  mm) with a capacity of 200 c.c. in which the level of blood is kept as constant as possible. A current of gas of the same mixture as that supplied to the lungs is passed continually through the small air space in the reservoir. Blood is taken by the pump from the bottom of the reservoir but as the blood must be shaken regularly to prevent sedimentation of corpuscles it is necessary to protect its exposed surface and for this purpose the use of liquid paraffin or mineral oil is much less convenient and efficient than the use of the gas mixture. From the pump the blood is passed through a heating coil maintained at  $38^{\circ}\text{C}$ , and thus to the kidneys, by way of the cannula in the aorta. Between the heating coil and the kidneys are placed, a manometer to register the perfusion pressure of the kidney, an air bubble catcher, and a T tube for the withdrawal of samples of blood. In the inferior vena cava is placed a cannula for the estimation of the rate of blood flow through the kidneys, and for the withdrawal of samples of venous blood for the estimation of its oxygen content.

*Artificial respiration* A mixture of gases containing definite percentages of oxygen, carbon dioxide and nitrogen is made and the lungs are inflated by the device described by Takeuchi(4). It may be worth while to note that the outlet on the stopper of the two way glass stopcock should be larger than the inlet in order to allow of as complete an expiration as is possible with the chest open. The inflation of the lung is controlled by water pressure of about 10 cm, the inflation should be quite small and should proceed at a rate of between 20 and 30 respirations per minute, a quicker rate will not allow of a complete collapse of the lungs.

*Filling the system with blood.* The system having been washed out with .9 p.c. saline is filled by placing 150 c.c. of blood in the reservoir and starting the pump. Air bubbles are prevented from collecting in the heating coil by inverting it while the blood is pumped through it. The output per minute is noted and the blood circulated in order to heat it to 38° C. The pump is now stopped and the artificial respiration started.

*The pump.* The pump (Fig. 2) consists of a modification of the original perfusion pump of Brodie and Dixon(5). The crank, which

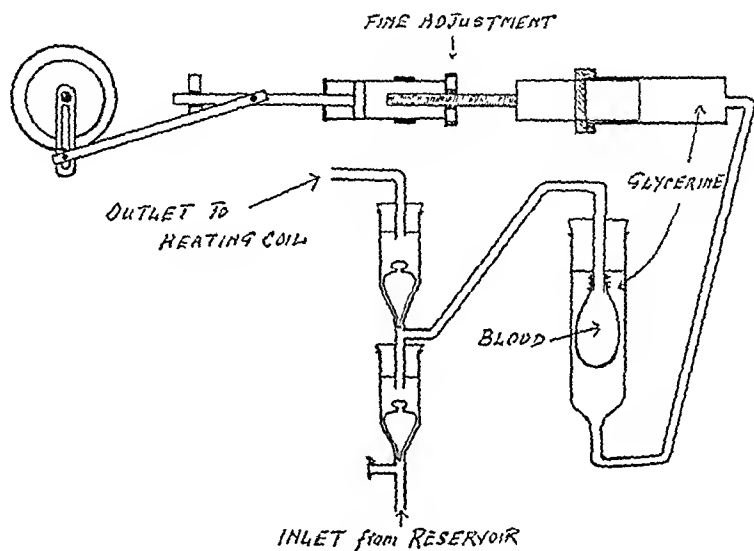


Fig. 2. Diagram of pump showing type of fine adjustment, valves and glycerine buffer.

gives a coarse adjustment is driven by a coned pulley. As the piston of the pump is long and works through a gland one is not troubled with leaks. The extent of the movement of the piston can be finely adjusted by a mechanism which consists of a cylinder through the upper orifice of which passes the driving rod attached to the crank. The end of this rod is provided with a collar, which fits loosely inside the cylinder, and when the collar reaches the end of the cylinder during the back stroke of the pump, the cylinder is drawn with it. The end of the cylinder which is attached to the piston rod has a screw of fine pitch, so that it can be moved easily and at any time to any position on the piston rod. Thus by altering the position of the cylinder on the piston rod, the thrust, that is the volume per stroke of the piston, can be finely regulated.

To prevent any contact of blood with the pump, a device described by Tribe(6) in 1914 was used. A stout glass cylinder was attached by

pressure tubing to the pump and the cylinder and pump filled with glycerine. To the tube leading from the valve mechanism was attached a small rubber bag which was placed in the cylinder and held therein by a large rubber stopper firmly inserted. With each movement of the pump blood was therefore drawn into and expelled from the bag. The glass valves devised by Dr Dixon were used.

It is important that the blood supply to the kidney be not cut off and to secure this end the kidneys are placed in the artificial circulation in the following manner. The clip on the superior mesenteric artery is removed, then that on the aorta, the pump started and the ligature previously placed around the aorta just below the superior mesenteric artery is firmly tied. The arterial blood pressure is maintained as constant as possible by the addition of blood by way of the jugular vein. The perfusion pressure figures are maximal for the pump, which was run at a rate varying between 35 and 40 strokes per minute.

The following results will show how effectively the artificially inflated lungs deoxygenate the blood.

TABLE I

	I	II
Bar pressure mm Hg	755.5	760.0
Gas mixture $O_2$ tension mm Hg	10	20
$CO_2$ " "	10	20
N " "	735	720
Normal blood $O_2$ p.c. saturation (1)	94.6	95.2
Deoxygenated blood $O_2$ p.c. sat (B)	22.0	43.4
Deoxygenated blood $O_2$ p.c. sat (C)	—	11.0
Venous blood from kidney $O_2$ p.c. sat (D)	5.3	26.0
Oxygen p.c. saturation from dissociation curve	20.0	10.0
Rate of blood flow through kidney in c.c. per min	40.0	30.0
Perfusion pressure in mm Hg	80.0	100
Arterial blood pressure in mm Hg	120	100

In calculating the oxygen content of the blood a correction must be made for the altered physical conditions, namely for the amount of oxygen and nitrogen taken up in physical solution by the blood between the temperature of the blood in the body and that in the bottle of the differential manometer.

#### ADDENDUM

*The reaction of the blood.* In most of our experiments the  $pCO_2$  was kept low in order to simulate a condition of anaemia accompanied by hyperpnoea. Such low tensions of  $CO_2$  in either oxygenated or reduced blood lowers the H-ion concentration of the blood, and therefore to determine to what extent the reaction of the blood has been altered a series of experiments on human and cat's blood was carried out by one of us (E. W. H. C.), the results of which are shown in Table II below.

With low oxygen pressures the H-ion concentration varies with the  $\text{CO}_2$  pressure, a  $p\text{CO}_2$  of 3 mm. Hg giving a  $p\text{H}$  of 7.98, while, as has already been shown by Parsons(7), if the  $p\text{CO}_2$  be maintained approximately normal a reduction of  $p\text{O}_2$  from 159 mm. to 8 mm. Hg results in a  $p\text{H}$  change of 0.07. It must be borne in mind therefore that in supplying the kidney with a blood of low p.c. oxygen saturation, that organ will have to function under conditions which will vary according to the method used for deoxygenation. If the deoxygenation be associated with hyperpnœa resulting in a washing out of  $\text{CO}_2$ , the kidney will be faced with a condition of alkalosis, while, on the other hand, if the desaturation be produced merely by lowering the oxygen tension, the  $p\text{CO}_2$  being normal, the kidney will have to deal with a blood the reaction of which is not appreciably altered.

TABLE II. Effect of alterations in  $p\text{CO}_2$  and  $p\text{O}_2$  on the  $p\text{H}$  and p.c. saturation of the blood.

$p\text{CO}_2$	Total $\text{CO}_2$	$p\text{O}_2$	$\text{CH} \times 10^{-3}$	$p\text{H}$	p.c. saturation
Cat's blood exposed to gas mixtures in the saturator.					
3.10	13.99	15.90	1.05	7.98	20.0
5.40	17.10	10.87	52	7.82	—
10.34	24.02	16.72	2.05	7.68	—
12.90	19.80	22.80	3.00	7.52	12.0
26.60	33.23	25.80	3.97	7.41	33.3
Human blood exposed to gas mixtures in the saturator.					
42.56	50.29	8.21	3.99	7.40	18.4
44.61	44.56	158.92	4.75	7.33	94.6
Cat's blood exposed to gas mixtures in the lungs.					
5.0	—	5.0	—	—	12.3
10.0	—	10.0	—	—	38.4
10.0	—	10.0	—	—	26.0
20.0	—	20.0	—	—	43.3
23.3	7.01	13.3	2.19	7.66	43.5

Thus for the supply of deoxygenated blood, the most suitable gas mixture is one containing  $\text{CO}_2$  and  $\text{O}_2$  at tensions approximately 40 and 5 mm. Hg respectively. If one's aim is to supply deoxygenated blood in which both the  $\text{O}_2$  and  $\text{CO}_2$  tensions are reduced, thus simulating an anoxæmic condition comparable to that produced by extreme hyperpnœa the gas mixture should contain  $\text{CO}_2$  and  $\text{O}_2$  at tensions of about 10 and 5 mm. Hg respectively.

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# STUDIES ON THE INNERVATION OF SMOOTH MUSCLE. II. On the Frequency of Nerve Impulses Entering and Leaving the Superior Cervical Ganglion.

By HARRY O. VEACH AND JAYME R. PEREIRA

*(From the Physiological Laboratory, Cambridge)*

INHIBITION of the smooth muscle of parts of the alimentary canal of the cat, by stimulation of the vagus nerve, resembles Widenky inhibition so closely(1) that the relation of the frequency of discharge from peripheral nerve cells to the frequency of stimulation of the pre ganglionic fibres assumes a special interest. The reactions of the alimentary tract indicate that this relation is a direct proportion. To obtain further evidence on the question, the present investigation was undertaken. It concerns the frequency of discharge from cells of the superior cervical ganglion of the cat in response to different frequencies of stimulation of the cervical sympathetic nerve, contraction of the mictitating membrane serving as indicator.

*Methods* Seven experiments were performed the results being uniform in all. Induction shocks, yielded by coils whose primaries were supplied with iron cores, were used for stimulation, the primary circuit being made and broken by rotary interrupters. A mercury-copper switch was placed also in this circuit. One of its arms was used to govern the duration of stimulation and to yield single makes and breaks, and the other was used to close the signal magnet circuit. In the first three experiments, the lower frequencies were supplied by an interrupter designed by Keith Lucas and described by Adrian(2), and the higher frequencies were given by a contact breaker described by Adrian and Olmsted(3). In the case of the latter, the duration of closure of the primary circuit was about 50  $\mu$ c. The former gave interruptions ranging from 1.03 to 31 per second when the circuit was made and broken through a contact operated by the cam wheel,  $D_2$ , in Adrian's figure, the interval between the make and the succeeding break shock being about 15 $\sigma$ . As a rule, however, the series of cam wheels,  $L$ , was used to interrupt the current. This yielded frequencies from 1.03 to 15.5 per second, the interval between make and break being about 30 $\sigma$ . In the last four experiments a new contact breaker similar to that described

by Adrian and Olmsted was employed. It differed from the latter, however, in this respect. One of the 16 conducting brass segments was extended laterally, beyond its fellows, in the surface of the fibre cylinder, so that with the proper position of the contact brush on the cylinder surface, only one contact per revolution was given. Its duration was about  $\cdot 032$  of that of a revolution. For interruptions from 2.5 to 32 per second, the single contact only was used, but for higher frequencies, the series of segments was employed. For the latter, the duration of closure of the primary circuit was about 48 p.c. The primary current was supplied by a 2-volt accumulator. For the purpose of obtaining thresholds, in the four experiments for which they are given, the same inductorium was used exclusively. This coil was used throughout in the last three of these, the primary current being adjusted to  $\cdot 09$  ampère. The number of interruptions of the primary current per second was taken as the frequency of stimulation, though both make and break shocks affected the nerve. The estimation was not considered accurate beyond the second figure. No extra resistance, other than that of the tissue stimulated, was placed in the secondary circuit.

Each cat received subcutaneously 0.75 to 1 gm. urethane per kilogram of body weight, and about one hour later, the anæsthesia was completed by chloroform. A tracheal cannula was inserted and the anæsthesia was maintained by a mixture of chloroform and ether when required. The animal's head was so adjusted that the nictitating membrane could be attached to the writing lever without friction of the connecting thread with the eye or the apparatus. Regularly the eyelids were cut off, and occasionally the contents of the eyeball were removed, to allow free movement of the membrane. A silk thread connected the middle of the free margin of the membrane with a small metal bar, cemented vertically and at right angles to a delicate lever near its fulcrum. This lever, to which a fire-polished glass writing point was attached by a flexible strip of paper, recorded the movements of the membrane on the kymograph paper. The magnification varied from 6 to 9.5 fold, and the load varied probably between the limits of 2 and 5 gm.

The cervical sympathetic was tied, cut, and isolated low in the neck for 3 to 4 cm. of its peripheral course. For stimulation, it was placed between two silver wire electrodes about 0.5 mm. in diameter and occasionally a glass shield(4) was used. The nerve remained in better condition, however, when it was kept covered with the tissues, being removed and placed on the electrodes only at times of stimulation.



After observations with the pre-ganglionic fibres had been completed, the post-ganglionic fibres, in all experiments except the first, were isolated and stimulated. The cervical sympathetic, for this purpose, was isolated up to the superior cervical ganglion; this ganglion was separated from the ganglion nodosum, and the post-ganglionic fibres were freed from their surroundings for several mm. A silk ligature was tied tightly about the nerve bundle at the cephalic end of the ganglion to cut off any possible effects of the ganglion cells. On one occasion, the pre-ganglionic fibres were faradized after this ligation, and there was no trace of contraction. The vagns was removed then from a point several mm. cephalad of the ganglion nodosum to a point several cm. caudad of it. To stimulate the post-ganglionic fibres, the electrodes were held peripheral to this ligature by the hand, and the nerve bundle was drawn between them. As a rule, the cathode for the break shocks was peripheral to the anode for both pre- and post-ganglionic fibres. In the case of the former, the electrodes were probably not more than 3 mm. apart, and for the latter, this distance was probably less than 2 mm.

The usual procedure of stimulation was the following. The threshold was first determined, the smallest contraction of the muscle detectable by the lever method being used as indicator. The usual frequency chosen for this purpose was 165 per second, though others were used at times, and the faradization was prolonged for 15 seconds unless contraction appeared sooner. The secondary coil was approximated then several centimetres from the threshold position toward the primary, to insure stimulation of all of the nerve fibres. The distance of approximation was usually about the same for both pre- and post-ganglionic fibres, and it was so chosen, as a rule, that the single break of the primary circuit caused distinct contraction. The nerves were then subjected to periods of faradization, usually about one second in duration, but occasionally considerably longer, and the corresponding contractions were recorded. Intervals of one to several minutes, depending on the time required for relaxation, separated the periods of faradization.

Special procedures involved in cooling the interior of the eyeball and the nictitating membrane, and in stimulating the nerve of the nerve-muscle preparation will be described in connection with the results obtained.

### *Results.*

All the data obtained on thresholds are given in the following table. In every instance, the threshold for the post-ganglionic fibres was decidedly higher than that for the pre-ganglionic. In Exps. 3 and 1,

moreover, a greater approximation of the secondary was required, in the case of the former, for the low frequencies of 10.6 and 11.8 than for 165. Single makes and breaks, from closing and opening the mercury-copper switch, also caused contraction, the latter being more efficient for both pre- and post-ganglionic fibres. Little effort was made to determine the minimal intensity of stimulus required for this effect, but in Exp. 2, distinct contraction occurred with a secondary position of 13 cm. for the right pre-ganglionic fibres, in response to the break, and at 7.5 cm. for the right post-ganglionic. The shocks became perceptible to the tongue with a position near 8 cm.

Exp.	Primary current (amp.)	Side stimulated	Frequency (per sec.)	Threshold (cm. distance of secondary)	
				Pre-ganglionic	Post-ganglionic
1	.14	Right	90	12.9	11.8
			136	—	11.5
2	.09	Right	165	13.9	11.5
		Left	165	13.0	10.6
3	.09	Right	165	13.6	12.2
		Left	165	13.0	12.2
			10.6	—	10.8
4	.09	Right	11.8	13.0	6.6
			165	13.3	7.5

The contractions evoked by stimulation of both pre- and post-ganglionic fibres with a given frequency are of quite the same character, provided that due account is taken of the difference in threshold. Thus as the stimulus interval is diminished in brief periods of faradization, either centrally or peripherally of the ganglion, the magnitude of the contractions increases, within limits, progressively (Figs. 1 and 2). The maximum, with periods of about one second duration, is usually reached at 140 per second. In the production of this steady augmentation, the following factors are involved. The latent period tends to decrease, and the rate of development of contraction to increase; as the frequency becomes greater (Figs. 1 and 2). The extent of the shortening during stimulation, therefore, is augmented. The excessive rise in the curve in Fig. 1, *F*, is probably due to the fact that the corresponding period of stimulation was somewhat longer than that immediately preceding and succeeding it. The continuation of the process of shortening on cessation of stimulation, furthermore, increases both in extent and duration with elevation in frequency. This after-action is a striking feature of the progressive augmentation in contraction (Figs. 1 and 2).

The similarity in the character of the response to stimulation of the pre- and post-ganglionic fibres with the same frequencies was illustrated

also in the few observations made with more prolonged periods of faradization. The duration of stimulation was usually about 30 or 60 seconds. For the low frequencies, viz., 1, 1.03 and 3 per sec., the contraction develops relatively slowly, and for the first two, slight elevations

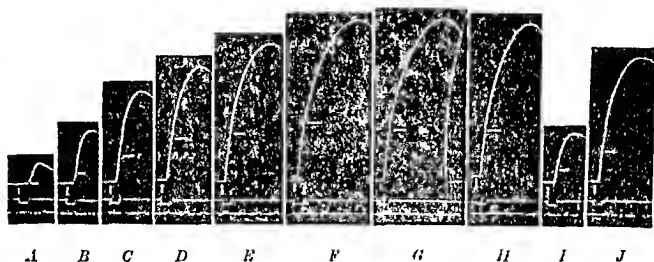


Fig. 1. *Exp. 4.* About  $\frac{1}{2}$  the original size. Right curve of sympathetic (pre-ganglionic) fibres and nictitating membrane. Position of secondary for observations, A to H, inclusive = 8.5 cm.; for I and J = 11.0 cm. Frequencies (interruptions per second): A = 2.95; B = 5.9; C = 11.8; D = 23.6; E = 47.2; F = 91.4; G = 142; H = 755; I = 472; J = 94.4. For this and Fig. 2 primary current = 0.9 ampere, magnification = 9.5; time is in 2 second intervals, and vertical and horizontal lines next to contraction curves indicate points on the latter corresponding to beginning and end of stimulation respectively.

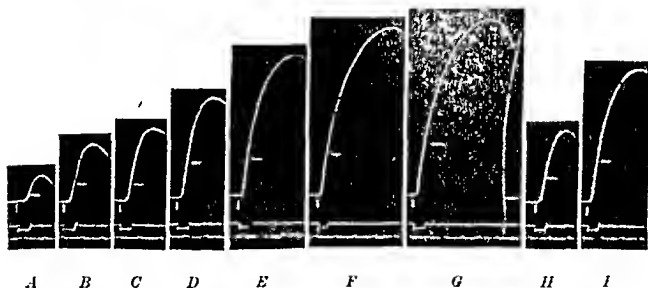


Fig. 2. *Exp. 4.* About  $\frac{1}{2}$  the original size. Right post ganglionic fibres and nictitating membrane. Position of secondary for observations, A to H, inclusive = 3.0 cm.; for observation, I = 0.0 cm. Frequencies: A = 2.95; B = 5.9; C = 11.8; D = 23.6; E = 47.2; F = 94.4; G = 142; H = 755; I = 755. Otherwise as for Fig. 1.

appear on the contraction curve, corresponding to each interruption. The shortening increases more rapidly during the first 5 to 10 seconds of

faradization, however, than later, when the curve tends to flatten. For the higher frequencies of 90 and 140, the contraction develops at first more rapidly, and it usually reaches a greater height than that produced by the lower. As faradization proceeds, however, the curve becomes much flatter, usually rising much more slowly in the later part of its course than that in response to the more widely separated stimuli. On cessation of stimulation with the different frequencies, the curve continues to rise or the muscle remains contracted, as a rule, for 1 to 2 seconds before relaxation begins. No pronounced difference in the contractions obtained thus, by stimulation of the pre- and post-ganglionic fibres with corresponding frequencies, was observed.

A further diminution in the interval between stimuli beyond that which suffices to yield the maximum contraction, in a series of brief periods of faradization, has one of two effects: the contractions are not much changed in magnitude, or a diminution occurs, becoming more marked the higher the frequency. If the strength of the stimuli is sufficiently great, the first result is obtained. It is a common occurrence, then, for frequencies up to 755 per second (the highest employed) to produce strong contractions on faradization of either pre- or post-ganglionic fibres (Figs. 1, *H*, and 2, *I*). In one experiment, the position of the secondary coil during a series of periods of stimulation of the post-ganglionic fibres remained unchanged, and the contraction in response to 595 per second was almost as great as that for 142. If the secondary is sufficiently far removed from the primary, however, the diminishing effect is well pronounced, as illustrated for the pre- and post-ganglionic fibres in Figs. 1, *I*, and 2, *H*, respectively. Thus in Exp. 3, a frequency of 94 produced pronounced contraction when applied to the left pre-ganglionic fibres. The shortening evoked by 380, however, was only about 0.7 as great, and 755 failed to produce a response. The position of the secondary for the three observations was 11.5 cm. A succeeding diminution in frequency, for either pre- or post-ganglionic fibres, under such conditions, evokes a stronger contraction. This effect is illustrated for the former in Fig. 1, *J*, and it was well marked for the latter, in Exp. 3, with a decrease from 189 to 142.

A striking difference occurs, when the diminution is pronounced, if the secondary is pushed toward the primary several centimetres and the nerve is faradized. The frequency which was formerly too high to produce strong contraction then causes a shortening of relatively great magnitude. The effect is illustrated in Fig. 2, *I*, the contraction often being relatively greater than that there shown. In Exp. 2, for example,

the contraction in response to a frequency of 575, and a secondary position of 5.0 cm, for the right post ganglionic fibres, was greater than that produced by either 94 or 188 with a position of 7.5 cm. Likewise, in the same experiment, faradization of the right pre ganglionic fibres at 377 per second and a secondary position of 6.0 cm, evoked a stronger contraction than 94 or 188 with a position of 9 cm. Similarly by weakening the intensity of stimulus, the effectiveness of a high frequency in evoking contraction is diminished for both pre- (Fig 1, I) and post-ganglionic fibres. The diminishing effects, described in this and the preceding paragraph, involve a diminution in the after action and in the rate of development of contraction, and usually an increase in the latent period. The augmentor effects, produced by increasing the intensity or decreasing the frequency of faradization, involve a reverse change in these factors.

In accordance with these results, alternately decreasing and increasing the interval between stimuli, in a single period of faradization, results in alternate diminution and augmentation in the extent of contraction respectively. For this reaction, however, the secondary coil must be sufficiently far separated from the primary, and the frequency must be sufficiently high. Furthermore, when partial relaxation has taken place as a result of the increase in frequency, strengthening the stimuli results at once in pronounced and well maintained contraction, as illustrated in Fig 3. These procedures were carried out only with the post ganglionic fibres.

In two experiments, an attempt was made to detect any modifying effect of the ganglion on the frequency of impulses passing through it by cooling the muscle of the membrane and its supplying nerve fibres. In the first of these, the pre ganglionic fibres only were stimulated, but in the other, the pre-ganglionic of the right side and the post-ganglionic of the left were subjected to faradization. A wide transverse incision was made into the eyeball and its contents were removed. In the first experiment, a stream of cold



Fig 3 Exp 2 Right post ganglionic fibres and mediating membrane. Primary current = 60 amperes. Beginning with down stroke of uppermost signal line secondary position = 9.8 cm, and frequency = 142 per second. At first stroke on middle signal line frequency increased to 283, and at second stroke, secondary pushed in to 8.5 cm. Time in 2.9 second intervals. Magnification 4.

Ringer, from a supply surrounded with an ice-water bath was directed against the medial part of the interior of the eyeball and allowed to flow out over the membrane for several minutes. The contraction in response to stimulation of the pre-ganglionic fibres, with frequencies of 11.8 and 188, was only diminished thus and not abolished. The interior of the eyeball was filled, therefore, with ice, which was allowed to remain for several minutes, with the same results. The characteristics of the diminished contractions, for both procedures, were much the same as those for the second experiment. In the latter, ice-water was substituted for the Ringer solution, and the contraction in response to stimulation of both pre- and post-ganglionic fibres, for periods of about one second, was greatly reduced. The latent period was much prolonged, contraction often appearing only after stimulation had ceased, and its rate of development was much diminished. For the pre-ganglionic fibres, cooling for five minutes reduced the contraction in response to 142 per second to about 5 p.c. of its preceding value. Interruption of the cooling for five minutes resulted in a marked increase in the height of contraction, and it was resumed, therefore, for seven minutes longer. The contraction in response to 142 was reduced about the same as before, and that for an immediately succeeding period at 11.8 per second was still smaller. This condition, however, was followed by gradual recovery. In the series of observations on the post-ganglionic fibres, moreover, 142 produced a weak contraction, immediately after cooling for five minutes, but a succeeding period at 11.8 per second caused no perceptible mechanical change. This observation was of interest in connection with the fact that the latter frequency had produced a contraction only about one half as great as the former before cooling was begun, the duration of stimulation in all instances being about one second. Gradual recovery was evident, however, on interruption of the cooling. Faradization of both pre- and post-ganglionic fibres, on the whole, gave much the same results.

*The cause of the diminished effectiveness of relatively high frequencies.* The increase in the effectiveness of faradization with relatively high frequencies, produced by approximation of the secondary to the primary coil, suggested at once that overlapping of induction shocks was the cause of the diminished mechanical response. To test the probability that the shocks were reduced thus to sub-threshold magnitude for the nerve fibres, two experiments were performed on the nerve-muscle preparation of *Rana temporaria* by the following method. It was placed in a moist chamber at room temperature, and the muscle was attached

to a recording lever. The nerve was drawn between silver wire electrodes, the cathode for the break shocks, in most of the observations, being placed peripheral to the anode. The new rotary interrupter was employed together with the inductorium used exclusively in the last five experiments on the nititating membrane. The primary current, supplied by a 2-volt accumulator, was kept constant at .09 ampère. No extra resistance, other than that of the sciatic, was placed in the secondary circuit. At the beginning of a series of observations, the threshold of the nerve was tested with a frequency of 5.9 per second, minimal contraction of the muscle being used as indicator. The nerve was then subjected to periods of faradization of progressively increasing frequency, and the threshold was tested for each. Succeeding the determination for each of these frequencies, however, the threshold was again determined for 5.9 in order that variations in excitability might be followed. In each observation, stimulation was continued for ten seconds unless contraction appeared sooner.

The excitability of the nerves gradually rose, during a period of about two hours after excision of the preparations, to a more or less

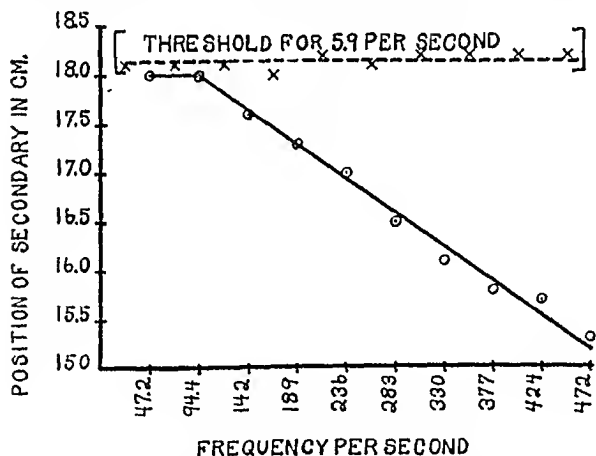


Fig. 1. Graphs illustrating the decrease in stimulating efficiency of the induction shocks as the frequency of interruption is increased above 94 per second. Stimulation of the sciatic of the nerve muscle preparation of Ranv. temp. Cathode for breaks peripheral to anode.

constant value. Both during the rise and the relative constancy, however, as the frequency was increased progressively above 94 per second, the effectiveness of the shocks diminished. It was necessary to approximate the secondary progressively nearer the primary, from the threshold position for 5.9, to obtain contraction. The results of a series of observations, during which the excitability remained quite constant, are plotted in Fig. 4.

This procedure has since been carried out with two other preparations, by one of us (V.), the duration of the shocks being shortened by removal of the iron core from the primary (10, pp. 412 and 413). The threshold position for frequencies up to 565 per second remained practically the same, under these conditions, as in the intercalated observations at 5.9. For the latter, however, a slightly greater approximation of the coils was usually required. Definite data for frequencies above 565 were not obtained. As an example of the constancy in the ability of the different frequencies to excite, the following table is given.

Frequency per sec.	...	5.9	189	5.9	283	5.9	377	5.9	472	5.9	566	5.9
Threshold (cm. distance of secondary)	...	...	11.1	11.3	11.0	11.3	10.8	11.3	11.0	11.3	11.2	11.3
Time (p.m.)	...	...	4.00	4.04	4.05	4.07	4.10	4.14	4.18	4.21	4.24	4.28

The ineffectiveness of a relatively high frequency, in contrast to the excitatory action of a lower frequency, is further illustrated in Fig. 5.



Fig. 5. Nerve-muscle preparation of *Rana temp.* Stimulation of sciatic with cathode for break shocks peripheral to anode. Intensity of primary current = .09 ampère. At beginning of faradization (uppermost signal line), position of secondary = 17 cm., and frequency = 472 per second. At first signal on lowermost signal line, frequency lowered to 47.2; at second signal frequency increased to 472, and at third, secondary pushed in to 13.5 cm. Time in 2 second intervals.



explanation. It appears from the work of Erlanger and Garrey (10) that overlapping may begin in the inductorium of the design used by Querido at frequencies as low as 54 per second. The occurrence of the effect more readily for the post-ganglionic than for the pre-ganglionic fibres, in Querido's experiments, probably has its explanation in the higher threshold of the former to induction shocks (cp. (11)).

The character of the diminished contraction obtained, when the frequency of faradization is sufficiently increased, is probably the resultant of two factors: (1) stimulation of only part of the nerve fibres, and (2) stimulation of at least some of these at a relatively low frequency. That the latter factor is involved is indicated by the similarity of such contractions to those produced with stimuli relatively widely separated. Irregularities in the magnitude of the induction shocks, with the higher frequencies of faradization, might lead to the occurrence of infrequent effective stimuli, which would produce a shortening of this sort. Evidence for this statement is given by the occurrence of separate twitches of the gastrocnemius in response to weak faradization of the sciatic with quite high frequencies.

The continuation of contraction of the nictitating membrane, on cessation of stimulation, is probably the result of the slow subsidence of a process set up within the muscle cells. The cells of the superior cervical ganglion are apparently not concerned in this after-action, for it occurs without decided change on stimulation of the post-ganglionic fibres. Experiments with nicotine (12), (8, p. 353, footnote), especially those of Langley, show quite conclusively, furthermore, that the pre-ganglionic fibres end about these cells. Inasmuch as the after-shortening is directly proportional in extent to the frequency of stimulation of the nerve fibres, within limits, and with brief periods of faradization, it is probably similarly related to the frequency of propagated disturbances delivered to the smooth muscle cells.

#### SUMMARY AND CONCLUSIONS.

1. The frequency of discharge from the cells of the superior cervical sympathetic ganglion of the cat is directly proportional to the frequency of reception from the pre-ganglionic fibres. It is probable, furthermore, that the propagated disturbances in passing through the ganglion are not altered decidedly in frequency (Figs. 1 and 2).

2. An erroneous impression of Wedensky inhibition may be derived from the diminished effectiveness of relatively high frequencies of faradization, when applied to either the pre- or post-ganglionic fibres

## ON CELLULAR CHANGES IN INTESTINAL FAT ABSORPTION. BY W. CRAMER AND R. J. LUDFORD.

*(From the Laboratories of the Imperial Cancer Research Fund.)*

IN the absorption of fat from the intestine a process of synthesis takes place in the epithelial cells lining the villi: the cells absorb the products of fat digestion—glycerine and fatty acids—and these products of hydrolysis are then made to unite again into neutral fats within the cells. Recent work on the Golgi apparatus has led to the conclusion that this structural element of the cell is particularly concerned in the specific functional activities of the cell, such as for instance, the formation of the specific secretory products. It seemed, therefore, of interest to determine whether the Golgi apparatus of the intestinal epithelial cells was concerned in this synthesis of fat during fat absorption.

In order to study this point it was necessary to use a method which would demonstrate at the same time the Golgi apparatus and the absorbed fat, in other words a method involving the use of osmic acid. Such a method was available in the Mann-Kopsch method for the Golgi apparatus in which the tissue is fixed in a mixture of sublimate and osmic acid. This was used in the modification recently described by one of us<sup>(1)</sup>. In using this method for the combined demonstration of fat and the Golgi apparatus it is essential to avoid an excess of fatty substances being present, which would reduce all the osmic acid in the fixing fluid so that none would be available for the demonstration of the Golgi apparatus. For this reason the amount of fat present in the food had to be reduced to a minimum.

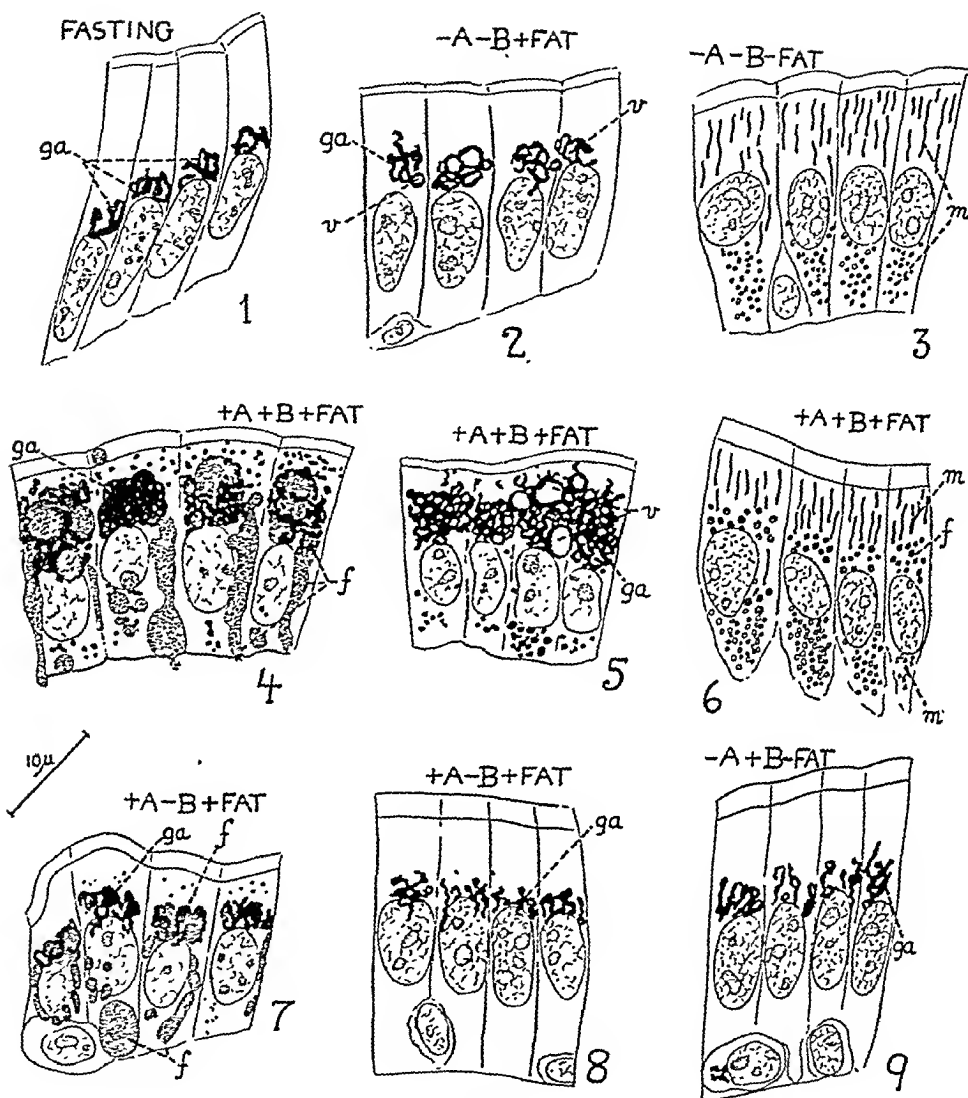
Another important point to be taken into consideration is the presence of vitamins in the food given. Mottram, Cramer and Drew<sup>(2)</sup> have shown that the presence of vitamins—especially of vitamin B—has a profound influence on the absorption of fat from the intestine. In the presence of these vitamins, fat is absorbed rapidly and efficiently as a stream of fine droplets running through the epithelial cells, and a large number of finely divided fat globules can be seen in the central core of the villus. In the absence of vitamins a few large globular masses of fat are found lying in the epithelial cells, between the free border and the nucleus, and no streaming of finely divided fat globules

takes place. The central core of the villus does not show many fat globules. This appearance suggests that the fat has been absorbed without having been split. It should be noted that these marked differences make their appearance in normal animals, and not merely in animals that have been kept for some time on a vitamin deficient diet. It may also be added that of the two vitamins, vitamin B is the more important one to ensure efficient and rapid absorption of fat.

In order to study the part played by the Golgi apparatus in fat absorption we have, therefore, compared this structure in the cells of a fasting animal with that in the cells of an animal fed on a diet rich in vitamins A and B. As a comparison, the Golgi apparatus was also studied in animals that had received a diet free from both vitamins A and B, and on diets free from vitamin B, and from vitamin A, separately. In practice, the experiments which were made on rats and mice were carried out as follows: mice were kept fasting for 24 hours, rats for 48 hours. They were then fed with a diet, the basal ration of which was the caseinogen starch salt mixture used in vitamin experiments. They were killed two and a half to three hours after feeding, and corresponding parts of the upper part of the small intestine were excised for fixation. Animals which received the basal diet only, which is free from both vitamins A and B and from fat, are described as - A, - B, - Fat. If marmite and cod liver oil were added the animals are described as having + A, + B, + Fat. If marmite alone was added the description is - A, + B, - Fat. If cod liver oil alone was added the description is + A, - B, + Fat.

The changes in the Golgi apparatus under these different conditions are illustrated in the accompanying figures.

Comparison of Fig. 1 of the Golgi apparatus in the fasting condition with Figs. 4 and 5, where in the presence of both vitamins, fat absorption is proceeding most actively, shows that in the active absorption and assimilation of fat by the intestinal epithelium the Golgi apparatus swells up and enlarges so as to form a network, nearly filling the part of the cell between the nucleus and the free border, with the globules of synthesised fat lying in its meshes. This change is very marked and as intense as any change hitherto described in the Golgi apparatus of other cells in different phases of cellular activity. The Golgi apparatus is much less altered in Fig. 2, where owing to the complete absence of vitamins in the diet the absorption and assimilation of fat are impaired. When only one vitamin is given fat absorption is more active than in the complete absence of vitamins, but less active than in the presence



Figs. 1-9.

Cytological changes in the epithelial cells of the intestine during the absorption of fat. (f. fat droplet; Ga. golgi apparatus; m. mitochondria; v. vacuole, the result of fat being dissolved out by treating the section with turpentine.)

Figs. 3 and 6 were drawn from Schridde preparations, the remaining Figs. from material fixed by the modified Kopsch method. The slides from which Figs. 2 and 5 were drawn were treated with turpentine, which removes the fat blackened by the osmic acid, but does not alter the blackened Golgi apparatus. Figs. 7 and 8 were drawn from different parts of the same section and show the variations in the form of the Golgi apparatus, dependent upon whether fat is actually being absorbed or not, irrespective of the fact that the diet is the same.

Attention is directed to Figs. 1, 4, and 5. Fig. 1 represents the Golgi apparatus in the fasting condition; Figs. 4 and 5 depict the change during active fat absorption and fat synthesis. For further details see text.

of both vitamins. The extent of the alteration in the Golgi apparatus depends upon the amount of fat absorption taking place. Conditions intermediate between the highest degree of fat absorption and its complete absence are correlated with relatively intermediate changes in the form of the apparatus, as is shown in Fig 7. Fig 8, which is from the same section as Fig 7, represents epithelial cells in which there happens to be no absorption and assimilation of fat. The appearance of the Golgi apparatus in the cells of Fig 8 resembles that of the fasting animal, showing that the swelling up of the Golgi apparatus is intimately associated with the process of fat absorption and fat synthesis by the epithelial cells. The same conclusion follows from experiments illustrated in Fig 9 in which an animal had received vitamin B but no fat. Here there is no absorption of fat owing to its absence from the diet, and the appearance of the Golgi apparatus is similar to that of the fasting animal (Fig 1). This indicates that the absorption of food material other than fat, by the epithelial cells is not associated with any change in the Golgi apparatus.

The mitochondria do not appear to take an active part in the process of fat absorption and assimilation. Figs 3 and 6 are drawings from preparations fixed by Schridde's bichromate osmic acid method in which the mitochondria have been stained red with acid fuchsin and thus differentiated from the black fat globules. Unless such a differential staining method is applied, the fat globules may be mistaken for mitochondria, and the erroneous impression may be gained that the long filamentous mitochondria, characteristic of the resting epithelial cells, are breaking up into granules during fat absorption. When properly stained, however, the appearances represented in Figs 3 and 6 are obtained. In the former there is no fat absorption, in the latter active fat formation has just commenced. A comparison of these figures shows that the mitochondria are not undergoing any morphological change during fat absorption. There may be, especially in the later stages, a decrease in their number, but it is difficult to discern the mitochondria in the later stages of fat absorption when the cell is filled with fat globules and therefore this decrease in number may be more apparent than real.

This point is of interest because after conditions of intense cellular activity the mitochondria are stated to decrease in number. Moreover, in some cases of yolk formation during oogenesis and in certain pathological conditions, the mitochondria have been said to swell up and become converted into fat. Our observations show that such a process does not occur in the absorption of fat by the intestinal epithelium. This is a

process of fat synthesis from its products of digestion and therefore quite different from those processes where fat appears as the result of a chemical degradation from more highly complex organic substances such as lipoids and proteins.

#### SUMMARY.

In the synthesis of fat, which proceeds in the cells of the intestinal epithelium during fat absorption, the Golgi apparatus is the cell structure mainly concerned.

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# TISSUE OXYGEN-TENSION WITH SPECIAL REFERENCE TO TETANY AND CONVULSIONS.

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*Effects of convulsions and tetany upon tissue O<sub>2</sub>-tension.* It was shown (1) that muscular exercise of moderate duration and severity and also insulin convulsions caused a marked rise of O<sub>2</sub>-tension in gas injected under the skin and into the abdominal cavity of a rabbit. Using the same technique, the effects of convulsions following intravenous injection of potassium cyanide and of strychnine have been studied. These drugs were injected slowly into an ear vein of an unanæsthetised rabbit in doses sufficient to produce definite convulsions, but at the same time, too small to cause either death or prolonged collapse. The results of examples of such experiments are given in Tables I and II. In the experiment illustrated in Table I, KCN M/100 in normal saline was injected in three doses of 1 c.c. each. After the first dose at the seventh minute, the animal exhibited obvious hyperpnœa; with the second dose at the 17th minute, the animal lay on its side for about two minutes

TABLE I. Rabbit 2 f kilo. Injection of KCN M/100.

Time (mins.)	Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg	
	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
0	55	25	51	37
7 1 c.c. KCN	—	—	—	—
17 "	—	—	—	—
27 "	—	—	—	—
83	10	28	31	45
121	36	28	38	45
384	43	32	13	49
21 hrs. later	50	27	51	40

TABLE II. Rabbit 2 15 kilo. Injection of strychnine hydrochloride.

0	43	22	43	10
30 0.1 mg. strychnine	—	—	—	—
46 0.12 "	—	—	—	—
123 0.1 "	—	—	—	—
223	36	29	37	15
320	38	31	10	43
19 hrs. later	46	29	46	10

exhibiting tetany-like tremors and at intervals kicking fairly strongly with its hind legs; following the third dose at the 27th minute, the animal again lay on its side; for about two minutes it made frequent running movements with its legs, licking sometimes with its hind legs; for a few seconds it kicked very violently and then its whole body became extended stiffly in opisthotonus; after a few seconds this passed off and the animal lay quietly on its side; at the 34th minute it had recovered sufficiently to sit up. It will be observed that the  $O_2$ -tension under the skin increased from 25 to 32 mm. Hg or by 28 p.c. whilst in the abdominal cavity it increased by 32 p.c., that is from 37 to 49 mm. On the other hand, the  $CO_2$ -tension decreased markedly, from 55 to 36 mm. Hg under the skin, and from 54 to 34 mm. Hg in the abdominal cavity. Five experiments of this nature were performed. Reference to the paper already mentioned (1) will show remarkable similarity between these results following KCN convulsions and those following muscular exercise. When numerous (*e.g.* 8 or 10) doses of KCN each in itself just too small to cause marked tremors or convulsions were injected at intervals over long periods, *e.g.* six or seven hours, no effect was produced upon the  $O_2$ -tension, but the  $CO_2$ -tension showed a decrease of about 7 or 8 mm. Hg, probably due to increased sensitivity of the respiratory centre to  $CO_2$ ; it is obvious therefore that the vigorous muscular contraction was responsible for the rise of  $O_2$ -tension.

Very similar results were obtained in five experiments with strychnine hydrochloride (see Table II). In the experiment illustrated 0.1, 0.12 and 0.4 mg. of strychnine hydrochloride in 1 c.c. normal saline were injected at the 30th, 46th and 123rd minute respectively. Tremors and fairly well marked convulsions lasting about two minutes and resembling those already described for KCN were produced after the 3rd injection. The animal made a very rapid recovery and seemed perfectly well again five minutes after the injection. As with KCN convulsions, the effects upon the  $O_2$ - and  $CO_2$ -tensions were very similar to those following muscular exercise. The  $O_2$ -tension increased by 41 p.c. under the skin, and by 12.5 p.c. in the abdominal cavity. As was sometimes observed with muscular exercise, the  $O_2$ -tension under the skin remained high for many hours after the convulsions. It was observed that when strychnine was injected every half hour for three hours in doses just too small to cause convulsions the  $O_2$ -tension was not affected, but the  $CO_2$ -tension was decreased by about 4 mm. Hg; it is thus obvious that as with KCN the rise of  $O_2$ -tension was due to the convulsions.

The effects of tetany following thyro-parathyroidectomy were next



studied. Four cats and four rabbits were employed; Dr H. H. Dale kindly performed the operations on the cats and Dr J. H. Burn those on the rabbits. Out of the eight animals operated upon, only two of them, both cats, developed typical tetany. One of the rabbits did exhibit some symptoms, not very definite, about three weeks after the operation; its appetite was poor and it became rather dull at first and then somewhat excitable; its hair lost tone and the animal was certainly in poor condition, but it recovered in a few days. At the time of this disturbance the rabbit exhibited changes in  $O_2$ - and  $CO_2$ -tensions similar, but less marked, to those described later for the two cats which developed typical tetany. These cats showed signs of the disease the day following the operation; on the second day, the tremors became marked and on occasion developed a convulsive character. One cat died four days after the operation and the other on the sixth day. As the symptoms produced and the changes occurring in the  $O_2$ - and  $CO_2$ -tensions in the tissues were similar, the full details for one cat only are given here (see Table III). The technique as regards the injection of gas and withdrawal of samples from the cats was similar to that used for rabbits; no anæsthetic was employed, and the cats were under observation for three weeks before the operation in order to obtain the normal figures for  $CO_2$ - and  $O_2$ -tensions under the skin and in the abdominal cavity. In two of the cats the  $O_2$ -tensions were much the

TABLE III. Cat 3.05 kilo. Thyro-parathyroidectomy.

Time (days)	Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg		Remarks
	$CO_2$	$O_2$	$CO_2$	$O_2$	
0	41	28	14	33	— —
4	43	32	13	31	— —
7	48	29	17	33	Operation
8	43	32	12	31	"Water shake" in fore paws
	40	32	15	33	Slight twitching
9	43	42	43	34	General twitching and tremors
	45	45	—	—	Tremors
	44	36	44	35	— —
10	43	44	44	40	Marked tremors, convulsive movements
	45	48	15	39	— —
11	40	42	41	41	Convulsive attacks
	41	41	38	41	— —
	—	—	—	—	Dead

same in both regions. This is exceptional; as a rule the  $O_2$ -tension in the abdominal cavity of a normal rabbit or cat was higher than that under the skin(2), but rare exceptions to this rule were observed with

rabbits as well as with cats. Female cats (parous) were used to obtain a larger abdominal cavity, male cats having a much smaller capacity for injected gas. In Table III it will be observed that following thyro-parathyroidectomy little change occurred in the  $O_2$ -tension until the tremors developed; but when these became marked and of a convulsive nature the  $O_2$ -tension under the skin was increased by 50 p.c., *i.e.* from an average of 32 mm. Hg before the operation, to a maximum of 48 mm., and in the abdominal cavity by 28 p.c., *i.e.* from an average of 32 mm. Hg before the operation to a maximum of 41 mm. On the other hand the  $CO_2$ -tension was hardly affected until a short time before death when the convulsive movements were most marked; then the  $CO_2$ -tension fell from an average of about 44 mm. Hg to 40 under the skin and from 44 mm. Hg to 38 mm. in the abdominal cavity. It is obvious therefore that the  $O_2$ -tension in the tissues was markedly increased at a time when the  $CO_2$ -tension was practically unaltered. This probably indicates that the cause of the rise of  $O_2$ -tension was not increased circulation in the tissues. Reference will be made to this again. The other cat which developed tetany exhibited an increase of 94 p.c., *i.e.* from 19 mm. Hg to 37 mm., in  $O_2$ -tension under the skin during the convulsive attacks. Its  $O_2$ -tension in the abdominal cavity was not estimated after the operation because at the critical moment no gas was found to be present.

The  $O_2$ -tension was therefore very definitely increased by the tetany, the increase lasting as long as the tremors and convulsions, that is for three days in one cat, and for four days in the other. The cause of the rise was therefore persistent and was probably acidosis as will be shown later. The animals which did not develop tetany exhibited no such changes.

By the above experiments it was proved that tetany and convulsions markedly increased the  $O_2$ -tension in gas injected into the abdominal cavity and under the skin. Great care has been taken to exclude the possibility that this rise of  $O_2$ -tension might be due to the presence of the injected gas. By previous experiment<sup>(1)</sup> it was shown that the increase following muscular exercise was quite independent of the volume of gas present and also of the absolute value of  $O_2$ -tension. Nevertheless even a small volume of gas might possibly by its presence irritate the adjacent tissues during the exercise so that hyperæmia might result. This has been negatived by experiments in which the injected gas was withdrawn entirely from the abdominal cavity and from under the skin before the exercise was performed. Then after the exercise had

been completed the same gas was put back again carefully under the skin and into the abdominal cavity at the same points whence it had been withdrawn. Care was taken to keep the gas at body temperature during the re-injection. It was found that in three such experiments exercise produced on an average a rise of 21 p.c. in  $O_2$ -tension and also a marked fall of  $CO_2$ -tension in the tissues. Controls were carried out at the same time, the procedure being exactly the same as the above but without the exercise; no effects were produced upon  $CO_2$ - or  $O_2$ -tension by the mere withdrawal and re-injection of the gas at the same point. In carrying out the experiments with muscular exercise it is advisable to choose active rabbits, so that fairly vigorous kicking exercise may be performed without unduly exhausting the animal; much exhaustion will lessen the characteristic effect. Care must also be taken when re-injecting the gas under the skin to inject it at the old place; injection under the skin at a point not previously injected will cause a rise of  $O_2$ -tension due to temporary hyperæmia, which passes off in a day or two(2). Experiments, six in number, have been carried out to prove that this hyperæmia was quite local, the rise of  $O_2$ -tension affecting only the region injected for the first time and not a distant and isolated region previously injected. During such hyperæmia the  $O_2$ -tension under the skin and in the abdominal cavity may be as high as 60 to 70 mm. Hg. This represents an increase of about 50 p.c. in the abdominal cavity and an increase of at least 100 p.c. under the skin, the normal figures when the hyperæmia has passed off being 30-40 mm. Hg in the abdominal cavity and about 20-30 mm. Hg under the skin. We may conclude then that a local inflammation might cause similar improvements in the local  $O_2$ -tension.

To return to the cause of the rise of  $O_2$ -tension following muscular exercise, tetany and convulsions, we may first consider the effects of certain conditions—which are produced by muscular contraction—upon tissue gas tensions. Muscular exercise causes an acidosis at least in the tissues; thus Barr and Himwich(3) have shown that lactic acid is removed from circulating blood during its passage through the arm after vigorous exercise with leg muscles. The tissues help to remove the acids from the blood to enable the blood reaction to remain normal. Rous and Drury(4) have shown that there may be a local acidosis in the tissues whilst the circulating blood shows no such change in reaction. Muscular exercise also causes an increase in circulation rate at least during the performance of the exercise and for a short period after its cessation; this increase of circulation rate may of course not involv

every region of the body. Also a rise of body temperature occurs as a result of muscular exercise. The effects of acidosis, of increase of circulation rate and of rise of body temperature upon  $O_2$ - and  $CO_2$ -tension in the tissues have therefore been studied. An acidosis was produced by injection of  $NH_4Cl$ ,  $CaCl_2$  and  $SrCl_2$  into an ear vein of a rabbit. J. B. S. Haldane<sup>(5)</sup> has shown that each of these salts when ingested in sufficient quantity causes an acidosis in man. Attempts were made to pass solutions of these salts into the stomach of an unanæsthetised rabbit by stomach tube, but the rabbit was so much disturbed that a considerable degree of muscular contraction resulted, sufficient in itself to cause some rise of  $O_2$ -tension in the tissues. The salts were therefore injected slowly into an ear vein of an unanæsthetised animal; with careful manipulation this method in itself caused no material disturbance of the animal. The salts were dissolved in normal saline solution, so that it was necessary to determine the effects—if any—of the injection of saline solution alone upon the  $O_2$ - and  $CO_2$ -tensions in the injected gas.

*Effects of intravenous injection of saline solutions and of water upon  $CO_2$ - and  $O_2$ -tensions in the tissues.* In seven experiments with water, tap or distilled, it was found that injection of much more than about 6 c.c. per kilo. and per hour, with a total of 30 c.c. in  $2\frac{1}{2}$  hours into a rabbit of 2 to 3 kilos. caused in some cases convulsions and death; similar results have been obtained by other researchers. Injection in non-fatal doses produced on an average in five experiments a fall of 5 to 6 mm. Hg in  $CO_2$ -tension and a fall of 3 to 4 mm. Hg in  $O_2$ -tension both in the abdominal cavity and under the skin. These results were probably due to laking of some of the blood and to disturbance of ions. On the other hand, as is well known, larger doses of 0.9 p.c. NaCl could be injected without harmful effect. Thus up to 100 c.c. were injected at body temperature in  $2\frac{1}{2}$  hours, that is 15 to 20 c.c. per kilo. and per hour. Except with quantities less than 5 c.c. per kilo. and per hour, or a total of about 25 c.c. in 3 hours definite effects were produced upon  $CO_2$ - and  $O_2$ -tensions in the tissues, the  $CO_2$ -tension being decreased and the  $O_2$ -tension increased; the effects were very marked with doses up to 13.8 c.c. per kilo. and per hour, that is a total of 100 c.c. in 3 hours. The results are given in Table IV and resemble those already given for muscular exercise. Similar but less marked results were obtained in eleven experiments with all doses down to less than 5 c.c. per kilo. and per hour. The results were probably due in part to increase of blood volume and of blood-pressure and probably also, as will be seen later,

to the Cl ion. It was obvious that the addition of NaCl to water converted a definite fall of  $O_2$  tension in the tissues into a definite rise. Glucose added to the saline did not modify the action of NaCl. In the conclusions reached later, allowance is made for any possible effects of normal saline itself in any experiments in which it was employed as the vehicle for other substances.

TABLE IV Rabbit 2.4 kilo Injection of 0.9 p.c. NaCl

Time (mins.)		Tensions under skin		Tensions in abdominal cavity	
		mm. Hg		mm. Hg	
		$CO_2$	$O_2$	$CO_2$	$O_2$
0		53	22	38	32
10	20 c.c. 0.9 p.c. NaCl	—	—	—	—
60		—	—	—	—
105	"	—	—	—	—
150	"	—	—	—	—
195	"	—	—	—	—
275		38	27	40	37
360		38	30	40	41

Experiments with hypertonic solutions of NaCl will be described in detail later. The smaller quantities of hypertonic solutions caused similar changes to those given in Table IV, but the larger doses produced a marked fall of  $O_2$  tension and eventually convulsions.

*The effects of experimental acidosis upon  $CO_2$  and  $O_2$  tensions.*  $NH_4Cl$ ,  $CaCl_2$  and  $SrCl_2$  in 5 or 10 p.c. solutions in 0.9 p.c. NaCl were injected in quantities which were too small to produce marked toxic effects. Four experiments were carried out with each salt. Tables V, VI, and VII record examples of such experiments. It will be seen that the effects of each salt upon the  $O_2$ - and  $CO_2$  tensions in the tissues were similar to one another and to those already given for muscular exercise, that is, there was a marked fall of  $CO_2$  tension and a definite rise of  $O_2$ -tension. Injection of similar quantities of 5 to 10 p.c. NaCl produced similar results. It seems likely therefore that the results were due at least in part to the Cl ion. It is not likely that the result was due to an increase of blood-pressure owing to increase of volume of circulating fluid owing to withdrawal of fluid from the tissues by these hypertonic solutions, it will be shown later that withdrawal of fluid from the tissues by strong NaCl solutions lowered  $O_2$  tension in the tissues. In the experiments with  $NH_4Cl$ ,  $CaCl_2$  and  $SrCl_2$  the animal exhibited symptoms of exhaustion for some minutes and a considerable degree of hyperpnea was produced. With large doses the animal became collapsed and the  $O_2$  tension fell whilst the  $CO_2$  tension still showed a marked fall. Urine was passed in some of the experiments.

TABLE V. Rabbit 3 kilo. Injection of 10 p.c.  $\text{NH}_4\text{Cl}$ .

Time (mins.)		Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg	
		$\text{CO}_2$	$\text{O}_2$	$\text{CO}_2$	$\text{O}_2$
0		—	—	—	—
2	1 c.c. 10 p.c. $\text{NH}_4\text{Cl}$	52	22	49	37
6	"	—	—	—	—
8	"	—	—	—	—
14	"	—	—	—	—
139		36	20	33	35
239		38	24	35	41
299		38	27	36	43

TABLE VI. Rabbit 2.55 kilo. Injection of 5 p.c.  $\text{CaCl}_2$ .

Time		$\text{CO}_2$	$\text{O}_2$	$\text{CO}_2$	$\text{O}_2$
0		55	19	52	33
5	5 c.c. 5 p.c. $\text{CaCl}_2$	—	—	—	—
47	"	—	—	—	—
110	"	—	—	—	—
187	"	—	—	—	—
257		40	19	38	36
407		40	24	36	42

TABLE VII. Rabbit 2.6 kilo. Injection 10 p.c.  $\text{SrCl}_2$ .

Time		$\text{CO}_2$	$\text{O}_2$	$\text{CO}_2$	$\text{O}_2$
0		54	18	52	33
15	5 c.c. 10 p.c. $\text{SrCl}_2$	—	—	—	—
70	"	—	—	—	—
145	5 c.c. 5 p.c. $\text{SrCl}_2$	—	—	—	—
265		39	18	35	42
415		37	24	35	42

It is not likely that increase in circulation rate by stimulation of the heart played any part in the rise of  $\text{O}_2$ -tension except in the case of  $\text{NH}_4\text{Cl}$ .  $\text{CaCl}_2$  in the doses used in the experiment in Table VI causes a fall of blood-pressure (Carlson and Jacobson(6)) and  $\text{SrCl}_2$  is a relatively inert salt. It is possible that  $\text{NH}_4\text{Cl}$  caused some alteration in circulation rate because in Table V each injection contained a smaller quantity of Cl than in the experiments with  $\text{CaCl}_2$  (Table VI) and  $\text{SrCl}_2$  (Table VII), yet the rise of  $\text{O}_2$ -tension was almost as marked with  $\text{NH}_4\text{Cl}$  as with the other salts; therefore the  $\text{NH}_3$  ion was possibly in part responsible for the rise. In the case of all these salts  $\text{NH}_4\text{Cl}$ ,  $\text{CaCl}_2$ ,  $\text{SrCl}_2$  and  $\text{NaCl}$  it is reasonable to conclude that the acidosis produced in the tissues by the Cl ion was responsible chiefly for the rise of  $\text{O}_2$ -tension, the increase in acidity aiding the liberation of  $\text{O}_2$  from  $\text{HbO}_2$  as it circulates in the capillaries of these tissues; it was suggested in a previous paper that lactic acid in muscular exercise produced a similar result(1). The marked fall of  $\text{CO}_2$ -tension was due chiefly to the removal of  $\text{CO}_2$  from the body by the effects of the Cl ion upon the respiratory centre.

*The effects of increase of circulation rate upon the  $\text{O}_2$ - and  $\text{CO}_2$ -tensions*

in the tissues. The effects of the commoner cardiac stimulants were determined by means of ammonia, ammonium carbonate, and caffeine. It has already been suggested that part of the rise of  $O_2$ -tension produced by  $NH_4Cl$  might have been due to the stimulating effect of the  $NH_3$  ion upon the heart. This was possible since intravenous injections of  $(NH_4)_2CO_3$  and of  $NH_4OH$  in suitable doses also caused a definite rise of  $O_2$ -tension, and a fall of  $CO_2$ -tension. When ammonia is administered either by inhalation or by mouth its stimulating action upon the heart is mainly reflex in origin. Injected slowly—1 c.c. per minute—into a vein  $(NH_4)_2CO_3$  (12 c.c. 3 p.c. solution) caused at first some temporary exhaustion, hyperpnœa and a slight fall of  $O_2$ -tension; this was followed by a rise of  $O_2$ -tension, the  $CO_2$ -tension being markedly decreased throughout. Indeed the details for the experiment with  $NH_4Cl$  in Table V would do just as well for this experiment with  $(NH_4)_2CO_3$ . It was obvious that any stimulant action the salt may have produced upon the circulation was complicated and masked at first by a temporary toxic action which caused the temporary exhaustion and the temporary fall of  $O_2$ -tension. Some experiments were also carried out with  $NH_4OH$  injected intravenously in 1 p.c. solution in normal saline. Of course, laking of blood must have occurred in this case but this may not have had much effect since it was proved that loss of 10 p.c. of the blood by bleeding had no effect whatever upon the  $O_2$ -tension in the tissues of a rabbit and caused only a small fall of  $CO_2$ -tension. 40 c.c.  $NH_4OH$  (1 p.c.) were injected into a rabbit of 2 kilos. in small quantities at intervals covering a period of 3 hours; there was no sign of exhaustion in this case and no temporary fall of  $O_2$ -tension but the  $O_2$ -tension subsequently increased by 30 p.c. under the skin and by 16 p.c. in the abdominal cavity, the  $CO_2$ -tension falling 20 p.c. in both situations. Larger doses of ammonium salts caused a marked fall of  $O_2$ -tension and eventually tetany and convulsions. As there was some doubt concerning the inter-

TABLE VIII. Rabbit 2.3 kds. Injection of caffeine.

Time (mins.)	Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg	
	$CO_2$	$O_2$	$CO_2$	$O_2$
0	53	33	56	36
11 35 mg. caffeine	—	—	—	—
111	46	35	49	35
151 35 mg. caffeine	—	—	—	—
246	44	39	51	35
281 40 mg. caffeine	—	—	—	—
391	42	41	46	42

pretation of the above results with ammonia and the salts, experiments were carried out with caffeine in stimulant quantities. Table VIII gives the results of an experiment.

Three doses of about 35 mg. caffeine were injected into an ear vein in a small amount of normal saline. The animal passed its urine on several occasions during the experiment. The  $O_2$ -tension was increased whilst the  $CO_2$ -tension in the tissues was decreased. We have thus definite evidence that stimulation of the heart in this way may cause an increase of  $O_2$ -tension in the tissues, obviously because of increased circulation. Strychnine which is sometimes stated to be a cardiac stimulant caused no effect upon  $O_2$ -tension unless the doses were sufficient to cause convulsions. These results have already been mentioned. It is obvious therefore that strychnine does not stimulate the heart in sufficient degree to affect the tissues so far as  $O_2$ -tension is concerned.

*Effects of rise of body temperature upon the  $CO_2$ - and  $O_2$ -tensions in the tissues.* To produce a rise of body temperature, the rabbit was overheated by radiant heat from a carbon arc lamp (70 volts, 30 amps.). The cage of the rabbit was placed on a level with the arc at about 2 feet distance. Five experiments were carried out in which the rectal temperature was increased by  $1.5^\circ C.$  on an average in a couple of hours. Such a marked rise of temperature was not observed with a few minutes' moderately severe muscular exercise unless this was performed under warm atmospheric conditions, *e.g.*  $24^\circ C.$  The rise of body temperature of  $1.5^\circ C.$  with the carbon arc caused on an average a fall of  $CO_2$ -tension of 3 to 4 mm. Hg under the skin and in the abdominal cavity probably owing to hyperpnœa. The  $O_2$ -tension in the abdominal cavity was not altered at all but under the skin there was a rise of  $O_2$ -tension of 3 mm. Hg perhaps due to dilation of vessels. It is evident therefore that rise of body temperature produced by muscular exercise could not have been the chief factor in the general rise of  $O_2$ -tension produced by muscular exercise, although it may have been of some influence as regards the  $O_2$ -tension under the skin.

*Discussion regarding the rise of  $O_2$ -tension following muscular contraction.* Fig. 1 gives a diagrammatic representation of the relation between the injected gas and the surrounding tissue of the subcutaneous region and peritoneal cavity. *A* represents a capillary of the tissue, *D* a cell (connective tissue of skin or epithelium of peritoneum), *B* the intercellular region with lymph or peritoneal fluid and *C* the injected gas. The tensions of  $CO_2$  in *C* were usually between 40 and 50 mm. Hg both under the skin and in the abdominal cavity whereas the  $O_2$ -tension



in *C* was about 30–40 mm. Hg in the abdominal cavity and 20–30 mm. Hg under the skin (2). The tensions in *B* were evidently the same as those in *C* with which it was in immediate contact. We do not know the  $\text{CO}_2$ -tension nor the  $\text{O}_2$ -tension in the cell *D* but they must be nearer to those in *B* and *C* than to those in *A*, the capillary. The blood in *A* is at first arterial; we know that the  $\text{O}_2$ -tension in arterial blood is between 90 and 100 mm. Hg. If we are to use figures for  $\text{CO}_2$ -tension in *A* and in *C* in our argument it is necessary to have exact information from simultaneous estimations of the  $\text{CO}_2$ -tensions in arterial blood and in *C*. A series of observations

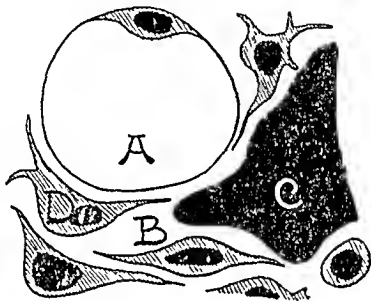


Fig. 1. For description see Text (W. J. P. del).

was therefore carried out in conjunction with my colleague Mr T. A. Webster. The  $\text{CO}_2$ -tension in the alveolar air was also estimated, using the Higgins-Plesch method as recommended by Henderson and Haggard (7), that is the rabbit (2–3 kilos.) rebreathed 100 c.c. of air by means of a mask and thin rubber bag for 20, 30 and 10 secs. The two estimations (1st and 2nd or 2nd and 3rd) which approximated the more closely to one another were averaged and this was assumed to give the alveolar  $\text{CO}_2$ -tension. The arterial  $\text{CO}_2$ -tension was estimated in blood withdrawn from a large artery in the ear. From a suitable animal it was possible to obtain 7–10 c.c. of blood in a minute or so, the blood flowing into the syringe under its own pressure. In only one animal (out of nine) was there any contraction of the artery sufficient to interfere with the withdrawal of the blood; the ear was warmed and a fine hypodermic needle was used. The syringe contained sodium fluoride and potassium oxalate and the estimation of the  $\text{CO}_2$  capacity of 1 c.c. plasma by Van Slyke's method was commenced at once. Then at

least two equilibrations were carried out, one at about 30–35 mm. Hg  $\text{CO}_2$  and the other at about 45 mm.  $\text{CO}_2$ . By estimations of  $\text{CO}_2$  capacities and by plotting in the usual way the  $\text{CO}_2$ -tension in arterial blood was obtained. The results are given in Table IX. It will be seen that the arterial  $\text{CO}_2$ -tension was very much lower than that in the injected gas in the tissues, on an average about 14 mm. lower.

TABLE IX.  $\text{CO}_2$ -tensions mm. Hg in eight different rabbits.

Arterial	Alveolar	In injected gas		When gas ( $\text{N}_2$ ) was injected under skin and into abdominal cavity
		Abdominal cavity	Under the skin	
35	38	56	56	4 days previously
35	39	49	51	6 " "
35	34	50	50	7 " "
33	37	48	51	9 " "
37	35	45	45	2 hours "
34	38	41	39	2 " "
32	35	44	43	4 " "
35	35	45	42	4 " "

It will also be observed that the alveolar  $\text{CO}_2$ -tension as estimated by the Higgins-Plesch method gave slightly higher figures than the arterial blood but the general agreement is good in this series of observations so that the method has come out better from this test than has often been the case; much criticism has been directed against the Plesch method, it being held that it may give even the  $\text{CO}_2$ -tension in venous blood. A comparison of the results for the alveolar  $\text{CO}_2$ -tension by the Higgins-Plesch method and those for the tissue  $\text{CO}_2$ -tensions reveals that the latter were on the whole much higher than the former but the difference was less marked when the gas had been injected only a few hours before. This was probably due to the temporary hyperæmia under the skin and in the abdominal cavity, which occurs for the first day or two following the injection of gas for the first time. Henderson and Haggard(7) and I myself in previous observations(2) stated that the alveolar  $\text{CO}_2$ -tension and that in injected gas in the abdominal cavity of animals—and under the skin in man and animals(2)—were similar to one another; in these former observations the  $\text{CO}_2$ -tension was estimated in the injected gas a few hours after injection, *i.e.* during hyperæmia, which probably explains why there was so close an agreement, the better circulation removing the  $\text{CO}_2$  from the tissue so that the  $\text{CO}_2$ -tension in the tissue approximated more closely to that of the arterial blood. We see, however, in Table IX that in normal animals when the conditions had settled down that the arterial  $\text{CO}_2$ -tension was much lower than that in the injected gas *C*, and therefore in *B*, of Fig. 1.

It is therefore certain that if the circulation in *A* is increased the  $\text{CO}_2$ -tension in *C* will be lowered and the  $\text{O}_2$  tension will be raised and of course if the circulation is decreased in *A* we shall get the opposite changes. It is also obvious that since the cell *D* uses up  $\text{O}_2$  and gives off  $\text{CO}_2$  any increase in metabolism of *D* will—other things being equal—lower the  $\text{O}_2$ -tension and raise the  $\text{CO}_2$ -tension in *B* and *C*, on the other hand, decrease of metabolism in *D* will raise the  $\text{O}_2$ -tension and lower the  $\text{CO}_2$  tension in *B* and *C*. These two conditions—namely increase of circulation and decrease of metabolism—must be excluded before we may assign the rise of tissue  $\text{O}_2$ -tension following muscular exercise to increased acidity of tissue. With regard to increase of circulation, the evidence we possess indicates that although the circulation rate is markedly increased during exercise it soon returns to normal on cessation of exercise. Henderson and Haggard(8) by their recent improved method of estimation of circulation rate in man found in one subject that the rate was lower following muscular exercise than at any other time. Evidence from blood-pressure records also indicates that although there is a rise of systolic pressure for a few minutes following exercise the pressure very soon falls to normal again (Cotton, Lewis and Rapport(9)). The pulse rate does remain in some cases slightly above normal for a considerable time but pulse rate is no definite indication of circulation rate, being more of an indicator of increase of body temperature and of distress of the heart. In man following exercise it is obvious that there is an increase of circulation in the skin due to overheating and sweating, and overheating undoubtedly plays some part in the rise of  $\text{O}_2$ -tension under the skin of a rabbit since we have already shown that a slight rise of  $\text{O}_2$ -tension was produced in that situation by a rise of body temperature, but there was no such rise of  $\text{O}_2$  tension in the abdominal cavity following overheating as there was following muscular exercise. Again it has already been pointed out that following parathyroidectomy the  $\text{O}_2$ -tension was markedly increased when the  $\text{CO}_2$  tension was unaltered in the tissues. This seems to exclude the possibility that increase of circulation was the main cause of the rise of  $\text{O}_2$  tension. The cause was obviously persistent following parathyroidectomy since the  $\text{O}_2$  tension remained high for days during the marked tetanic contractions; lactic and other acids were probably being produced continually.

With regard to metabolism we know that there is a definite rise of  $\text{O}_2$  consumption for some considerable period following muscular exercise (Benedict and Cathcart(10), A. V. Hill and Lupton(11)). Benedict

and Cathcart pointed out that this increase may be present without any corresponding increase in lung ventilation; that is, there was a greater liberation of  $O_2$  from  $HbO_2$  by the tissues which was due probably to increased acidity of the tissues. It is obvious that a fall of metabolism could not have been the cause of the rise of  $O_2$ -tension under consideration unless this fall was localised to the regions under the skin and in the abdominal cavity.

The increase of  $O_2$ -tension was evidently due to some factor or factors which persisted for some period following the muscular exercise; that lactic acid was not removed from the tissues for some considerable period is certain; so that increase of acidity fits the case well enough from the time point of view. Increase of hydrogen ion within the limits possible during life dilates the blood vessels. This may also play some part, but a general dilatation of vessels by itself will lower the  $O_2$ -tension owing to a fall of blood-pressure, *e.g.* after histamine(8).

It is likely that hyperpnœa played some part in the rise of  $O_2$ -tension following exercise. In most cases, but not in all, *e.g.* parathyroidectomy, the  $CO_2$ -tension in the tissues fell markedly at the same time that the  $O_2$ -tension increased. It is likely that, although the  $CO_2$ -tension did not fall markedly in the tissues following tetany of parathyroidectomy, nevertheless the  $CO_2$ -tension in the arterial blood was reduced owing to acidosis, as shown definitely by Cruickshank(12). Probably the rate of flow of blood was not sufficient to remove the  $CO_2$  from the tissues fast enough to cause a fall of  $CO_2$ -tension therein; the rise of  $O_2$ -tension was—at least in this case—independent of circulatory changes.

The fall of  $CO_2$ -tension was undoubtedly due chiefly to the rapid removal of  $CO_2$  from the body following exercise, hyperpnœa being obviously present owing to the effects of increase of acidity upon the respiratory centre. It is well known that the  $CO_2$ -tension in arterial blood may remain below normal for an hour or more in man following muscular exercise indicating persistence of non-volatile acids. Excessive hyperpnœa by itself(1) causes a fall of  $O_2$ -tension in the tissues owing to alkalosis; but in exercise or experimental acidosis there cannot be an alkalosis. Hyperpnœa plus acidosis is probably the ideal combination for increase of  $O_2$ -tension in the tissues, the hyperpnœa increasing the  $O_2$ -tension in the lung alveoli and therefore in solution in the blood and the tissue acidosis accelerating the liberation of  $O_2$  from  $HbO_2$ . I carried out five observations on the  $O_2$ -content of arterial blood taken from an artery of the ear of a rabbit before and immediately after exercise, and found 13.7 volumes p.c. before

exercise and 14.6 volumes p.c. after exercise, indicating a slight increase in the  $O_2$  content of arterial blood which was probably due to hyperpnoea. Thus hyperpnoea may have played some small part in the increase of  $O_2$  tension following exercise because hyperpnoea did not pass off immediately.

It is concluded that the rise of  $O_2$ -tension under the skin and in the abdominal cavity following muscular exercise, tetany or convulsions is due chiefly to acidity of tissue and in part to rise of body temperature, the main factor, as previously suggested (1) apparently being increased liberation of  $O_2$  from  $HbO_2$  in the tissues.

*The cause of tetany and convulsions* Tetany and convulsions occurring in different conditions have been attributed to different causes, e.g. asphyxial convulsions to combined  $O_2$  lack and  $CO_2$ -excess, parathyroid tetany to deficiency of Ca (Collip (13)), strychnine convulsions to increased excitability of the central nervous system and so on. The similarity of the phenomena have led several observers to look for a cause common to all, their theories have been reviewed recently by Critchley (14).

Collip and Clark (15) have found that guanidine still causes tetany though the blood Ca is high, so that if there is a common cause for tetany it must be some factor other than deficiency of Ca. Morris (16) has put forward the theory that tetany produced in a variety of ways is caused by deficiency of  $O_2$  in the blood. He studied the  $O_2$  content of the arterial and venous blood. Now I have studied the  $O_2$  tension a step nearer to the cell and I have found that a number of conditions—excessive hyperpnoea, injections of certain doses of guanidine,  $NaHCO_3$  (1),  $Na_2SO_4$ , NaCl, insulin, histamine, alcohol, water, ammonium salts and also anoxaemia (17)—which eventually give rise to tetany or convulsions caused a marked fall of  $O_2$  tension in the tissues. Greenwald (18) found that large amounts of NaCl or of  $Na_2SO_4$  caused tetany and I have confirmed this. I found that if NaCl (or  $Na_2SO_4$ ) be injected rapidly tetany and convulsions were produced very soon but if NaCl (or  $Na_2SO_4$ ) were injected more slowly a considerable fall of  $O_2$  tension was observed in the tissues before the tetany was produced (see Table X). The fall of  $O_2$  tension may have been due to withdrawal of water from the tissues, Weed and McKibben (19) have shown that NaCl produces marked shrinking of tissues.

Morris (16) and others have proved that alcohol causes tetany and I have observed that alcohol also markedly lowered the  $O_2$  tension in the tissues (see Table XI).

TABLE X. Rabbit 3.0 kilo. Injection of 11.6 p.c. NaCl.

Time (mins.)		Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg	
		CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
0		43	20	41	40
15	10 c.c. NaCl (11.6 p.c.)	—	—	—	—
65	"	—	—	—	—
82	"	—	—	—	—
115		40	21	38	41
129	10 c.c. NaCl (11.6 p.c.)	—	—	—	—
216		40	20	38	37
250	10 c.c. NaCl (10.0 p.c.)	—	—	—	—
295		42	15	34	31
320	10 c.c. NaCl (10.0 p.c.)	—	—	—	—
373		43	11	33	26

TABLE XI. Rabbit 2.1 kilo. Injection of 10 p.c. alcohol.

0		47	31	47	37
15	10 c.c. C <sub>2</sub> H <sub>5</sub> OH	—	—	—	—
45	"	—	—	—	—
85	"	—	—	—	—
135	"	—	—	—	—
165		38	27	38	32
175	10 c.c. C <sub>2</sub> H <sub>5</sub> OH	—	—	—	—
250		33	24	33	26
277	10 c.c. C <sub>2</sub> H <sub>5</sub> OH	—	—	—	—
328		34	20	—	—
398		35	18	35	28
18 hrs. later		48	25	48	33

The deficiency of O<sub>2</sub> in the tissues of the skin and in the abdominal cavity must be regarded as holding elsewhere. I would suggest that the cause of tetany and convulsions is deficiency of O<sub>2</sub> in the central nervous system. It is true I have not found a decrease of O<sub>2</sub>-tension in the tissues in the preliminary stages of KCN poisoning, of strychnine poisoning or of tetany following parathyroidectomy; but KCN may be regarded as causing a deficiency of O<sub>2</sub>-tension in the cells, by a direct action upon them; it is well known that the cell, although O<sub>2</sub> is present, behaves in KCN poisoning as if it were in an atmosphere of N<sub>2</sub>. It is not unreasonable to suppose that strychnine poisoning and parathyroidectomy interfere with the intracellular oxidative processes in some similar manner. In the case of parathyroidectomy perhaps we do not need to assume such a factor since there is evidence (12, 20) that tetany is due in this case to alkalosis and, as I have shown (1), conditions causing alkalosis markedly lower the O<sub>2</sub>-tension in the tissues; I failed however to observe such a fall after parathyroidectomy, perhaps because tetany followed too rapidly in my experiments.

*Teleology of tetany and convulsions.* That tetany and convulsions following parathyroidectomy, insulin, etc., improve the condition of the

animal is well known; and this in spite of the fact that during a convulsion, respiration is stopped and  $\text{CO}_2$  accumulates, thus leading to some weakening of the heart. I have shown above that tetany and convulsions increase the  $\text{O}_2$ -tension in the tissues and I suggest that tetany and convulsions have the same aim as hyperpnœa, namely to increase the supply of  $\text{O}_2$  to the tissues including those of the central nervous system. It is admitted that there are conditions of  $\text{O}_2$ -deficiency *e.g.* fainting, where muscular relaxation is produced and not convulsions; in such cases there is apparently a paralytic factor which would of course prevent tetany or convulsions.

There is evidence that breathing  $\text{O}_2$  at increased tension (below 70 p.e.) is of benefit in many conditions<sup>(21)</sup> which cause tetany and convulsions; it must be pointed out that breathing  $\text{O}_2$  at very high tension, above 70 p.e., will itself cause convulsions owing to the production of pneumonia (Lorrain-Smith<sup>(22)</sup>), which interferes with the passage of  $\text{O}_2$  through the lung.

It has been proved above that acidosis increased the  $\text{O}_2$ -tension in the tissues; it is therefore to be expected that administration of acids would be of value in tetany; there is evidence<sup>(20, 23)</sup> that this is the case.

#### SUMMARY.

1. Tetany and convulsions following parathyroidectomy, KCN poisoning and strychnine cause a marked rise of  $\text{O}_2$ -tension in the tissues, similar to that produced by insulin convulsions and muscular exercise. Strychnine and KCN in repeated doses just too small to cause convulsions have no effect on  $\text{O}_2$ -tension.

2. Many conditions which eventually give rise to tetany or convulsions cause a marked fall of  $\text{O}_2$ -tension in the tissues.

3. It is suggested that tetany and convulsions are caused by  $\text{O}_2$ -deficiency in the cell (brain, spinal cord) and that the purpose of tetany and convulsions is to counteract this defect.

4. Injection of water intravenously causes a fall of  $\text{O}_2$ -tension in the tissues; addition of small quantities of NaCl to the water causes a definite rise of  $\text{O}_2$ -tension.

5. Intravenous injection of  $\text{NH}_4\text{Cl}$ ,  $\text{CaCl}_2$ ,  $\text{SrCl}_2$  and NaCl in certain doses causes a marked rise of  $\text{O}_2$ -tension and a marked fall of  $\text{CO}_2$ -tension in the tissues; these effects are probably due to acidosis.

6. Injection of small doses of ammonia or salts of ammonium and stimulant doses of caffeine cause a rise of  $\text{O}_2$ -tension and a fall of  $\text{CO}_2$ -tension in the tissues, probably owing to increased circulation.

7. Rise of body temperature (by  $1.5^{\circ}\text{C.}$ ) causes a definite fall of  $\text{CO}_2$ -tension in the tissues and a rise of  $\text{O}_2$ -tension in the skin.

8. The  $\text{CO}_2$ -tension in the arterial blood (rabbit) is much lower than that in gas, injected some days previously, under the skin and in the abdominal cavity. The difference is much less a few hours after the injection.

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# THE SECRETION OF SWEAT AND VASO-DILATATION PRODUCED BY PILOCARPINE. By J. H. BURN, M.D.

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## *The vaso-dilator action of pilocarpine.*

IN an earlier paper (1) it was shown that, though the effect of denervation on the sweating response of a kitten's foot to pilocarpine was not constant, the change in this reaction was parallel to the change in the vaso-dilator reaction of the denervated leg to histamine. During the accentuation of the sweating produced by pilocarpine, usually seen in the earlier days after nerve section, the vaso-dilator reaction to histamine was abnormally pronounced; when, as usually happened after a few weeks, the pilocarpine effect became abnormally slight or disappeared, the vaso-dilator response to histamine was correspondingly reduced or annulled. It should be noted that the plethysmographic records of the vaso-dilator response were made on the kitten under ether, while the action of pilocarpine on sweating was tested without anaesthesia. A possible explanation of the relation was that pilocarpine might itself have a vaso-dilator action of a kind similar to that of histamine. Experiments have therefore been carried out (a) in which plethysmograph records have been taken of the limbs of anaesthetised cats, (b) in which the effect of pilocarpine has been observed on the perfusion of isolated limbs of cats and dogs, the opportunity being used of a series of such perfusions carried out together with Dr H. H. Dale for another purpose, in connection with which the apparatus used will be described in a forthcoming paper.

Fig. 1 shows the result of a plethysmograph experiment on a cat from which the right stellate ganglion had been removed 5 weeks before. The intravenous injection of .05 mgm. pilocarpine caused a fall of blood-pressure accompanied by a conspicuous dilatation of the right fore limb, and by very slight dilatation of the left fore limb. Fig. 2 shows the effect of .05 mgm. pilocarpine injected into the arterial cannula carrying the blood to the isolated leg of a dog. The animal's own defibrinated blood was used as perfusion fluid, and a constant pulsatile pressure was maintained. The injection of pilocarpine produced a sharp increase in limb volume and in venous outflow, while the perfusion pressure fell

slightly owing to the rapid escape of blood from the reservoir through the leg.

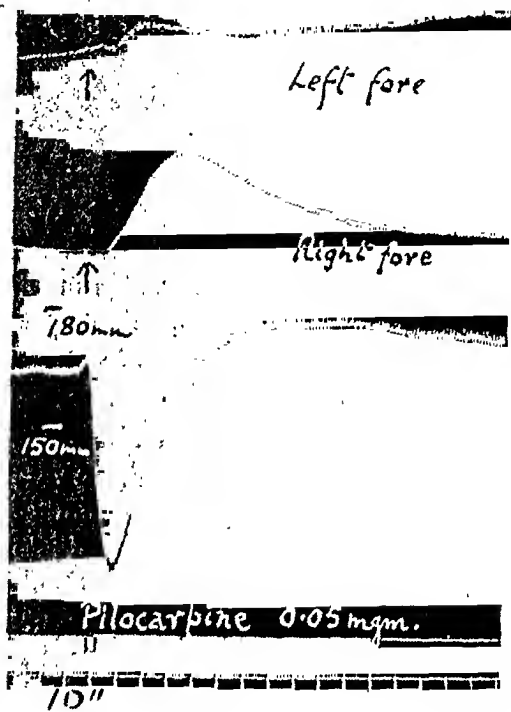


Fig. 1.

Fig. 1. Dilatation of fore limbs of cat with 0.05 mgm. pilocarpine. See text.



Fig. 2.

Fig. 2. Upper tracing—expansion of volume of perfused limb of dog with pilocarpine. Middle tracing—perfusion pressure. Lower tracing—venous outflow.

Fig. 3 shows the effect of similar injections of pilocarpine into the perfused hind limb of the cat. In the isolated limb of this species it is unusual to observe a dilator effect. The first tracing in the figure shows a dilatation of the limb volume which, though small, is significant because of the simultaneous increase of venous outflow. The second tracing, obtained in the same experiment a few minutes later, shows the absence of dilatation following the injection of histamine, and the third the absence of effect following the same dose of pilocarpine as before. Acetyl-choline, however, still produced a striking expansion of the limb volume and an increased outflow.

The experiments show not only that pilocarpine has a true, peripheral vaso-dilator action, but that this action closely resembles that of

histamine, and differs from that of acetyl-choline, in that it seldom survives under the conditions of artificial perfusion in the cat's limb, though

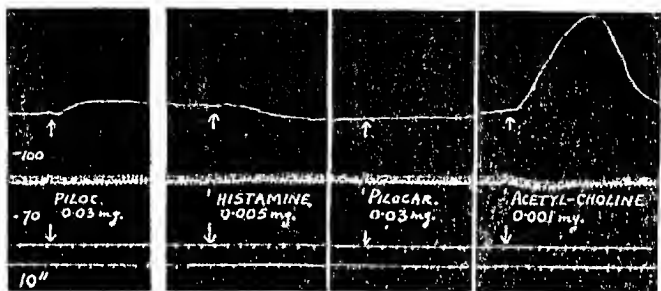


Fig. 3. Perfused leg of cat. Records as in Fig. 2. See text.

regularly in that of the dog. This characteristic difference between the cat and the dog will be considered, in the case of histamine, in the subsequent paper. Where the histamine effect persists, that of pilocarpine persists also, and both appear to fail together. The conclusion seems justified that the vaso-dilator action of pilocarpine is exerted on the same part of the vascular apparatus as that of histamine, that is to say, in the cat, mainly on the capillary vessels. The correspondence, previously observed, between sweating response to pilocarpine and vaso-dilation to histamine acquires a clearer meaning, when it is recognised that the vaso-dilator action of pilocarpine itself shows a similar, and indeed, an even closer correspondence to its sudorific action, as will be seen later.

#### *Effect of root section on sweat secretion.*

In the work previously described (1) it was shown that the usual disappearance of the sweat response to pilocarpine, following the complete denervation of a limb, was not due to the degeneration of the sympathetic fibres. Since no other nerve supply to the sweat glands is known, and since the loss of the sweat response was accompanied by the loss of the vaso-dilator response of the whole limb to histamine, it was suggested that the loss of the sweat response was secondary to this vascular change, which was assumed to be due to the degeneration of the sensory fibres. At the suggestion of Dr Dale, this hypothesis has been tested by experiments on four cats in which the nerve roots have been divided.

The operations have been carried out in each case by Dr Dale under deep ether anæsthesia, with full aseptic precautions.

*Division of posterior roots between ganglia and cord.* In two cats, the 4th, 5th, 6th, 7th lumbar and 1st and 2nd sacral posterior roots were divided on the right side, the anterior roots being preserved, so that the limb retained the power of voluntary, though ataxic movement.

Exp. 1. On the 3rd and 6th days after the operation 1.4 mgm. pilocarpine (s.c.) produced more sweating on the normal than on the operated hind paw. On the 6th day after operation the cat was excited and made angry. Pronounced sweating was seen on the normal hind paw, but only traces on the operated paw. On the 14th day 0.2 mgm. adrenaline was injected into the ear vein; this produced general sweating on the three normal paws, and occasional beads on the operated paw. On the 4th and 6th days the surface temperature of the operated paw was less than that of the normal paw.

On the 21st and 29th days pilocarpine produced the same sweating on both paws; on the 21st the surface temperature of both paws was the same. Seven months later, pilocarpine produced conspicuously greater sweating on the operated paw, and the surface temperature of this paw was above that of the normal.

Exp. 2. Cat 3.8 kgm. Diminished sweating to pilocarpine and diminished surface temperature of the operated paw were observed on the 3rd and 5th days.

The sweating to pilocarpine and the surface temperature of the two hind paws were identical from 1 to 6 weeks.

Plethysmograph observations were made under ether on the 45th day, and the animal killed before recovery. The sciatic nerves were divided on both sides. When .01 mgm. histamine, .001 mgm. acetyl-choline and .05 mgm. pilocarpine were injected into the anæsthetised animal (i.v.), all three substances produced the same dilatation in both legs. The reaction to pilocarpine is shown in Fig. 4.

*Excision of posterior root ganglia together with adjacent portion of the anterior root.* Exp. 3. Cat 3.5 kgm. The operation was carried out on

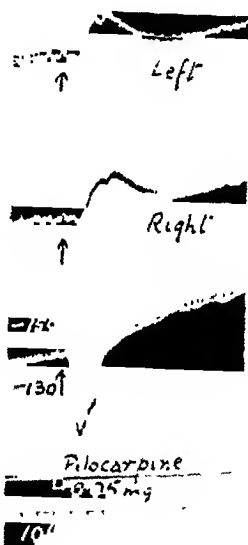


Fig. 4. Hind limbs of cat with lumbar and sacral posterior roots divided on right side. Dilatation with pilocarpine.

the 5th, 6th, 7th lumbar and 1st and 2nd sacral roots of the right side, the right hind leg being thus completely paralysed.

From 2 to 7 weeks 1.5 mgm. pilocarpine (s.c.) produced profuse sweating on the normal, but little or none on the operated paw. On one occasion (19th day) anaesthesia was induced with ether; this produced sweating on the normal, but not on the operated paw.

In a plethysmograph experiment on the 50th day, under ether, faradisation of the respective sciatic nerves, after these were divided,

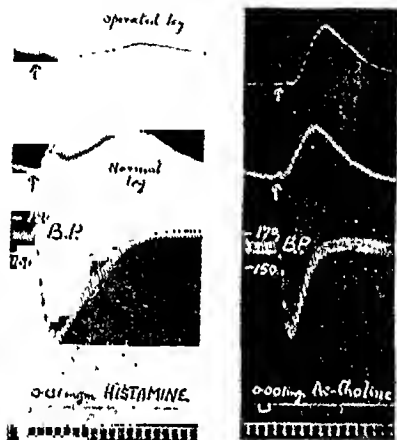


Fig. 5. Cat. Lumbar and sacral posterior root ganglia and adjacent portion of anterior roots removed on right side. See text.

evoked sweating on both paws. The intravenous injection of .01 mgm. histamine caused dilatation of the normal, but not of the operated leg; the injection of .001 mgm. acetyl-choline caused dilatation of both legs (see Fig. 5). After death, the removal of the ganglia and the integrity of the sympathetic path, were verified by dissection.

*Division of posterior and anterior roots.* Exp. 4. Cat 3.2 kgm. These were divided, leaving the ganglia intact, by an intradural operation on the right side from the 4th lumbar to the 2nd sacral inclusive.

On the 21st, 25th, 31st days, 2 mgm. pilocarpine caused profuse sweating on the normal hind paw, but only traces on the operated paw.

The pads of this cat were unpigmented and when the normal paw secreted the pad flushed; the operated paw, which did not secrete, remained pale.

On the 35th day, under ether, the vagi and both sciatic nerves were divided and the hind limbs placed in plethysmographs. The injection of pilocarpine (0.05 mgm.) produced dilatation of the normal, but *no effect* on the operated limb (see Fig. 6). The injection of histamine, however, caused dilatation in both legs, though the expansion began earlier and lasted longer in the normal leg. Acetyl-choline caused the same dilatation in both legs. The general result of these experiments is set out in Table I.

The experiments give further support to the earlier evidence that the changes in the sweat response to pilocarpine are due to changes in the limb vessels, for in each case they were accompanied by corresponding changes in the dilator action of either pilocarpine or histamine. In the case of Exp. 4, the dilator effect of pilocarpine disappeared together with the secretory action, but the dilator effect of histamine was only slightly diminished.

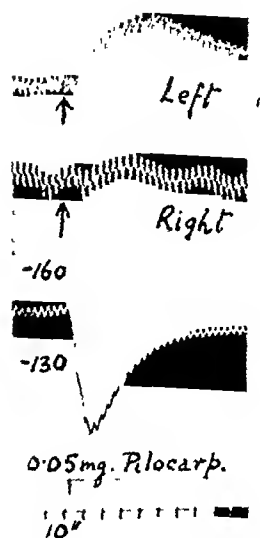


Fig. 6. Disappearance of dilator response to pilocarpine in right hind leg after degeneration of only the motor nerves to the voluntary muscles.

TABLE I.

Operation	Degeneration	Sweat response to pilocarpine	Dilator effect of		
			pilocarpine	histamine	acetylcholine
Section of post. roots	None	Unchanged	Normal	Normal	Normal
Excision of post. root ganglia and adjacent anterior root	Sensory and motor fibres	Greatly diminished	(Not observed)	Greatly diminished	Normal
Division of post. and ant. roots	Motor fibres	Greatly diminished	Abolished	Slightly diminished	Normal

This is an exception to the observations previously made that the dilator effect of histamine was absent in limbs in which pilocarpine produced no secretion.

The observations in Exp. 4 disprove the suggestion that the sensory fibres are responsible for the vascular condition on which the sweat

response to pilocarpine depends. In this cat, although both sensory and sympathetic fibres were intact, and only the efferent fibres to the voluntary muscles degenerated, both the secretory and dilator actions of pilocarpine were lost in the operated leg.

The experiments also show that the dependence of the activity of the sweat glands on the vascular conditions is not confined to the case in which the stimulus is pilocarpine. In Exp 1, six days after division of the posterior roots, sweating produced by excitement of the animal was much less on the operated side. On the 14th day, the sweating evoked by the injection into the ear vein of a large dose of adrenaline was seen on both hind feet, but very much more on the normal than on the operated paw. It has previously been shown<sup>(1)</sup> that this effect of adrenaline is probably due to central stimulation by the rise of arterial pressure, for it is entirely absent if the sympathetic path is interrupted. In Exp 3, sweating was produced by administering ether, although the sympathetic fibres of both sides were intact, it was only seen on the normal paw. It may be supposed that in all these three cases, the same stimulus to secretion passed from the centres to the pad of each hind foot. Because the vascular conditions were less favourable on the foot of the operated side, the stimulus produced less secretion on that foot than on the normal foot.

*The exaggeration of the sweat response after degeneration  
of the sympathetic fibres*

It has been recorded<sup>(1)</sup> that the usual effect of extirpation of the stellate ganglion of one side in the kitten is to exaggerate the sweat response to pilocarpine. A similar phenomenon has been described by Takakusu<sup>(2)</sup> in the rabbit, in which, after extirpation of the superior cervical ganglion of one side, pilocarpine produces a greater salivary secretion. Two explanations of the exaggeration of the sweat response are possible. After degeneration of the secretory innervation of the glands, it may be that the cells are hypersensitive to pilocarpine as the denervated pupil is to adrenaline. On the other hand, as the degeneration of the secretory fibres is accompanied by degeneration of the constrictor fibres to the vessels, the denervated paw may have a better blood supply, and, because of this, may respond with more secretion when pilocarpine enters the circulation. If this second explanation be correct, the exaggeration should be greater the cooler the surroundings for then the blood supply to the normal paw will be smaller, and the difference in the supply of the two paws greater.

Careful observations to test this point were made on one kitten. As it was difficult to estimate the total quantity of sweat, the time of appearance of visible sweat on the two paws was recorded, for sweating always appeared earlier on the paw which secreted most. After the kitten was placed in a room at  $37^{\circ}$  for an hour, the sweat response was simultaneous in the two paws. At lower external temperatures, however, the sweat always appeared first on the denervated paw, and the interval grew longer the lower the temperature of the air. The observations are plotted in Fig. 7. It was also observed that while the surface temperature of

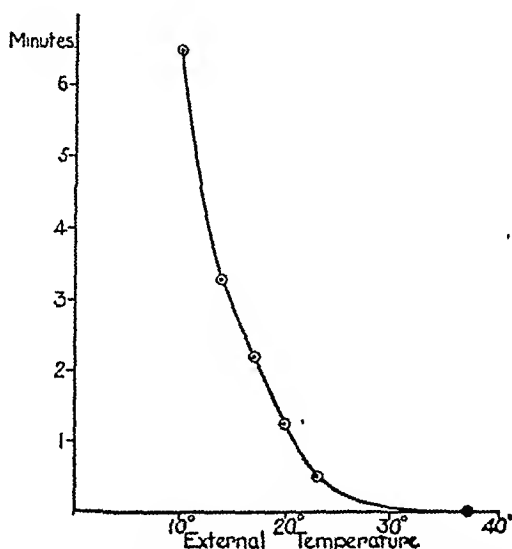


Fig. 7. See text. The external temperature at which the observation was made is recorded as abscissa, and the interval by which the sweat on the denervated paw anticipated that on the normal paw is plotted as ordinate.

the denervated paw varied very little with the external temperature, that of the normal paw fell rapidly as the kitten was placed in cooler air. The temperatures observed were:

External temp.	Temp. of paw without sympathetic fibres	Temp. of normal paw	Difference
23°	28°	28°	0°
16	28	26	2
14	28	24	4
12	26	20	6
4	25	14	11

There was evidently a relation between the difference in the surface temperature of the two paws and the interval by which the sweating



on the denervated paw anticipated that on the normal paw. The acceleration produced by denervation appears to be due to the better blood supply of the denervated paw at ordinary conditions of external temperature. In two other kittens, however, it was seen that even at 37°, when the restriction of the blood flow to the normal paw must have been minimal, sweating regularly appeared earlier on the denervated paw. In these cases it must be supposed that the denervation had caused also a true exaggeration in the response of the sweat glands to pilocarpine, comparable to the paradoxical reaction of the denervated pupil to adrenaline.

*The dilator reaction to histamine in the normally innervated leg.*

In the previous paper it was stated that the correspondence, observed in all denervated limbs, between the sweat response to pilocarpine and the dilator effect of histamine was not seen in the case of normal limbs. Usually the dilator response of such limbs to histamine, as seen under ether, was insignificant, although the sweat response to pilocarpine, without anaesthesia, was considerable; an example of this discrepancy is seen in Fig. 1. When it received an injection of pilocarpine, the cat sweated more on the paw without sympathetic fibres than on the normal paw, but the difference appeared to be only about 2 to 1. When the cat was anaesthetised with ether, however, the difference in the dilator response of the two limbs to pilocarpine appeared to be about 10 to 1. It was possible that the small dilator effect in the normal limb was due to a vascular change produced by the anaesthetic, and the following experiment was carried out to test this.

Exp. 5. Cat 2 kgm. 1.5 gm. per kgm. urethane (s.e.). After 4 hours, during which the cat was kept warm, tracheotomy was performed, ether being given in addition during the operation. The volume of one fore limb was recorded by plethysmograph, and a cannula was inserted into the femoral vein for injection. The effect of injecting .01 mgm. histamine on the limb volume was then alternately observed under urethane alone and under urethane together with ether. The ether was administered by putting the open end of the trachea tube into a Wolff's bottle containing ether, without adjustment of the side opening of the tracheal cannula, so that there was no change in the amount of oxygen and carbon dioxide in the respired air. The result is shown in Fig. 8. Under urethane and ether the injection of histamine caused no dilatation of the limb volume; actually the volume diminished. The trachea tube was then removed from the ether bottle. After 2 to 3 minutes, it was observed

that the limb volume and the pulsations in the limb steadily decreased. When a steady state was reached an injection of histamine produced a

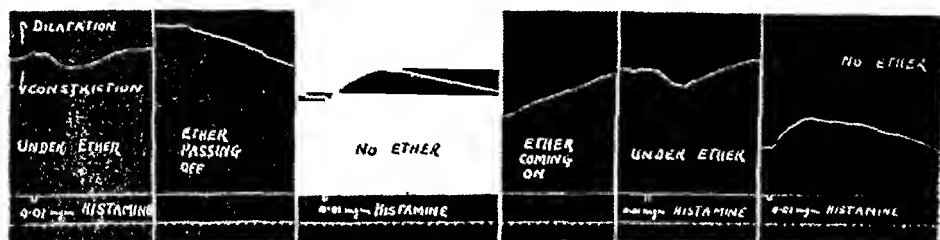


Fig. 8. Record of volume of fore limb of cat anesthetized with urethane. When ether was breathed in addition, histamine caused only a diminution of the limb volume; under urethane only histamine caused dilatation. Note the diminution of limb volume as ether leaves the circulation, and the increase as it enters.

good dilatation. Ether was again administered; the limb volume expanded and the pulsations increased in size; again, the injection of histamine produced only diminution of limb volume. These observations were repeated four times with the same effect.

It was clear that ether was able to abolish the dilator effect of histamine in a normal limb<sup>1</sup>. In limbs in which only sympathetic fibres had degenerated the dilator effect was always large under ether, so that the abolition in a normal limb must have been effected by way of the sympathetic fibres. This point was confirmed by an experiment similar to the one just described, on a cat from which the right stellate ganglion had been removed, and in which the limb volume of both fore limbs was recorded. In Fig. 9 it is seen that under urethane alone both limbs dilated when 0.01 mgm. histamine was injected; the dilatation in the denervated was greater than that in the normal limb, but the disparity was comparable with the disparity between the sweat responses of the two limbs to pilocarpine. When ether was administered in addition to urethane, the dilator effect of histamine in the normal limb disappeared, while in the denervated limb it was unaffected. Following the removal of the ether the dilator effect in the normal limb returned.

These experiments account satisfactorily for the failure to observe in normal legs a dilator response to histamine corresponding to the sweat response to pilocarpine. Had urethane been used as anæsthetic instead

<sup>1</sup> It should be noted that the dilator responses of the normal limbs shown in Figs. 4, 5 and 6 were observed immediately after section of the sciatic nerve. The effect of ether on the dilatation was consequently excluded in these cases.

of ether, in the earlier plethysmograph experiments, the correspondence between the two reactions would presumably have been complete in all

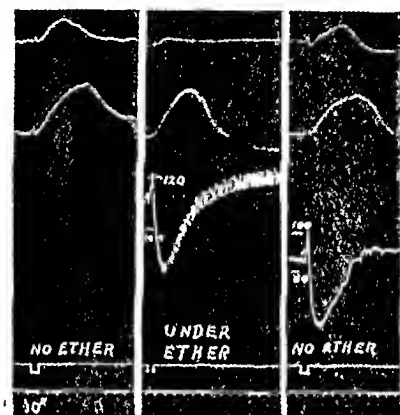


Fig. 9. The upper tracing shows volume record of left fore leg of cat with intact nerve supply. The middle tracing shows right fore leg with sympathetic fibres degenerated. At signal, 0.1 mgm. histamine (i.v.) in each case. Under urethane only both legs dilate; under urethane and ether, dilatation does not occur in the left fore leg.

cases. The experimental result in Fig. 8 brings out the further point that the administration of ether causes a peripheral dilatation by way of the sympathetic nerves. The simplest explanation of this is that ether causes central diminution of vaso-constrictor tone. Ether, however, is known to be a central stimulant of the sympathetic; thus it causes sweating, salivation, and a discharge of adrenaline from the suprarenal glands. The alternative, therefore, presents itself that the dilatation observed in the above experiment may be due to active dilator impulses passing down the sympathetic. Since the result is to reduce or abolish the vaso-dilator effect of histamine, leaving that of acetyl-choline unpaired, it appears that the effect, whatever its nature, is produced in that part of the vascular mechanism where histamine produces its dilator effect.

#### Discussion.

It has been shown that, in the cat, pilocarpine possesses a vaso-dilator action on the vessels of the limbs qualitatively resembling and

about one-fifth as strong as that of histamine. It has also been shown that after complete denervation of a limb, the diminution of the sweat response of the hairless pad to pilocarpine is accompanied by the diminution of this vaso-dilator reaction. This correspondence, founded on plethysmograph experiments, is borne out by the colour changes in the feet of cats with unpigmented pads. Sweating evoked by pilocarpine on a normal pad is accompanied by flushing; when, as a result of denervation, the pad of the opposite side does not sweat, neither does it flush. The dilatation produced by pilocarpine in a normal limb must result in a greater amount of pilocarpine reaching the sweat glands of that limb than reaches the glands of the denervated limb which does not dilate, and the diminution of the sweat response in the denervated limb may be wholly accounted for in this way.

It has long been recognised that sweat secretion is closely dependent upon the temperature of the skin and on its blood supply, and in Exps. 1 and 3 the equal central stimulation of the glands of both hind paws caused by excitement, adrenaline and ether caused less secretion on the paw which was colder and had more contracted blood vessels.

The experiments show further that the vascular changes after denervation are not due merely to degeneration of the vascular nerve supply itself. The dilator and secretory effects of pilocarpine disappeared in Exp. 4 in which both sympathetic and sensory fibres were undegenerated. The disappearance accompanies the degeneration of the motor nerve fibres to the voluntary muscles, and seems to be a consequence of the effect of muscle atrophy on the circulation of the leg. This conclusion helps to explain the varying results after sciatic section previously recorded, for after this operation a good deal of the leg musculature receives its normal innervation. In this condition the persistence of a sweat response to pilocarpine probably depends on the extent to which activity in the still normally innervated muscles compensates for the effect of the denervated muscles on the circulation. The explanation is peculiarly suited to the case of kitten 10 recorded in the previous paper(1). After section of the sciatic nerve, an exaggerated pilocarpine response persisted during  $10\frac{1}{2}$  weeks' observation. After section of the brachial plexus, the response on the fore paw disappeared within a few days of the operation. The kitten was a healthy and active animal whose movements were scarcely affected by sciatic section on one side; all the normally innervated muscles of the hind leg were in constant use, and it may be supposed that the persistent dilator response to histamine and sweat response to pilocarpine were due to the efficient circulation

through the foot preserved by their activity. On the other hand, section of the brachial plexus paralysed all the muscles of the fore leg. As a consequence the sweat response to pilocarpine and the dilator effect of histamine disappeared and severe trophic changes were observed in the skin.

The evidence of the part played by muscular activity in the maintenance of a normal circulation through the skin provides an experimental basis for the value of massage and passive movement in the clinical treatment of denervated limbs.

The diminution of the sweat response to pilocarpine on the foot of the cat following degeneration of the motor nerves to the leg muscles can have no relation to Horsley's clinical observation (4) of the failure of pilocarpine to produce sweating in areas innervated from below a transverse cord lesion. Pembrey (3), moreover, records that paraplegic cats sweat on both hind feet in response to central and peripheral stimuli. In Exps. 1 and 2, however, it was found that early after section of the lumbar posterior roots of one side there were intervals of 3 weeks and 5 days respectively, during which the sweat response to pilocarpine on the corresponding hind foot was conspicuously diminished. Later, in each case, the sweat response recovered. There was no degeneration of the voluntary or involuntary motor nerves, but during this early period after the operation there was very little movement of the leg, and the impoverishment of the circulation, further demonstrated by the lower surface temperature, was probably related to the disuse of the muscles. It is possible that Horsley's observations are to be explained in the same way.

Experiments are described which demonstrate that the exaggeration of the pilocarpine response after degeneration of the sympathetic fibres is in large part due to the removal of the constrictor supply to the blood vessels. It may be that these observations supply the clue to certain paradoxical findings of Knauer and Billigheimer (5). These authors describe two cases of patients suffering from war neurosis, in which spontaneous sweating was seen in the early morning confined to one-half of the body, when pilocarpine was injected later in the day, sweating appeared only on the opposite side. That spontaneous sweating occurred on one half of the body may be taken to mean that a greater number of impulses travelled down the sympathetic fibres of that side, impulses presumably not confined to the secretory fibres of the sweat glands, but passing down the vascular fibres also. If this unilateral, exaggerated sympathetic tone persisted to any extent throughout the day, then the

skin of that side would have a poorer blood supply than that of the opposite side. My experiments indicate that under such conditions the injection of pilocarpine might be expected to produce sweating more readily on the side of the body on which the spontaneous sweating did not occur.

#### SUMMARY.

1. Pilocarpine has a dilator action on the vessels of the cat and the dog, closely resembling that of histamine.

2. After denervation of the leg of a cat, the sweat response of the hairless pad to pilocarpine persists only so long as the vaso-dilator response of the whole leg persists.

3. The loss of the sweat response and of the dilator response to pilocarpine after denervation is not due to degeneration of the sympathetic or sensory nerve fibres, but to degeneration of the motor fibres of the leg muscles. The loss is probably a consequence of muscular inactivity and atrophy.

4. It is shown that the amount of sweat secretion produced by a given central stimulus depends on the blood flow through the pad of the foot.

5. The exaggeration of the sweat response to pilocarpine caused by degeneration of the sympathetic fibres is shown mainly to be due to the removal of the sympathetic control of the blood vessels, but also (in the case of some kittens at least) to a hypersensitiveness of the denervated glands and vessels to pilocarpine.

6. It is shown that ether exerts a dilator effect on the vessels of the cat's limb by way of the sympathetic nerves. This dilator action is produced on those vessels which are dilated by histamine.

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# THE RÔLE OF THE PHOSPHATES IN CARBOHYDRATE METABOLISM IN SKELETAL MUSCLE.

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## PART I

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PHOSPHORIC acid is present in the muscle in two forms at least, namely free as a mixture of mono- and di-basic phosphates, and in organic combination with a hexose, probably fructose diphosphate or lactacidogen. The quantitative relationship between the two forms is a somewhat variable one, but, as will be seen later, in normal fresh muscle for a particular animal (rabbit, cat, dog or frog) the ratio between the two varies within narrow limits. Even when muscle is strongly stimulated until fatigue ensues, and is then quickly fixed, the ratio in many animals (dog and frog especially) may be but slightly different from that in resting animals. In this respect the hexose ester differs from the glycogen in muscle.

There are evidently two processes concerned in the maintenance of this well-defined ratio, one hydrolytic, the other synthetic. As regards the former, the process takes place rapidly when the muscle is kept for some time after its removal, and especially if it be minced and warmed. In order to effect a complete hydrolysis, the minced muscle is kept in a weak sodium bicarbonate solution at 45° for 1-2 hours (Embsen and Adler(1)). As regards the synthetic process, it is evident that the maintenance of the ester form within the living muscle at so constant a level, and the rapidity with which free phosphate entering muscle is

transformed in part into the organic form, suggest the action of some such esterifying ferment as has been suggested by Euler and his school(2) as playing a part in alcoholic fermentation. Euler thus refers to a hexosephosphatase or hydrolytic ferment, and a hexosephosphatase, the esterifying one. Definite evidence of synthesis or esterification has been brought forward by Embden and Lehnartz(3) from a study of the action of different anions on minced muscle or expressed muscle juice. Of these anions much the most active is the fluoride one which may, especially in the presence of added glycogen or starch, almost completely synthetise the store of free phosphate. Among the organic anions which favour synthesis, the one of greatest physiological importance is the lactate, which at the same time favours the breaking down of glycogen. Both acids which are formed during muscular activity, phosphoric and lactic, are able to bring about the mobilisation of the necessary carbohydrate for the formation of the hexose ester. According to Weber(4) there is no evidence of a building up of glycogen from the action of these anions which aid in the synthesis of the ester; usually however they check the breaking down of the polysaccharide, while the ions which facilitate hydrolysis of the ester, *e.g.* the  $\text{Cl}^-$  and  $\text{HCO}_3^-$ , increase glycogen decomposition. The phosphate and lactate supplement one another in their action on glycogen. It is to the action of the ions, especially the chloride, which hasten the process of hydrolysis, that the recuperative effect of these salts on muscle rendered inexcitable by the action of isotonic cane sugar is due. The evidence therefore brought forward by Embden, Lehnartz and others shows the important part played by different anions on the phosphate metabolism.

A similar investigation from the same school by Lange(5) deals with the influence of cations, especially calcium, on the same metabolism. He concludes that the typical  $\text{Ca}^{++}$  effects are: an increase in the breaking down of glycogen and in the formation of lactacidogen and a check in lactic acid production and in oxidation. All these effects, with the exception of the first, are to be observed, under the action of the fluoride, and, as will be seen later, even the glycogen continues breaking down under the influence of this ion, but not to the same extent as under chloride. In attempting to arrive at a knowledge of the way in which these various effects are produced, Embden has drawn special attention to the hydration of the muscle colloids produced by the anions or cations which aid the hydrolysis of the ester, and the dehydration of the same colloids by the ions which favour esterification.



This investigation deals with the phosphate metabolism in skeletal muscle obtained from animals with a normal carbohydrate metabolism and also in muscle taken from animals showing hyper- or hypo-glycaemia. In Part I normal metabolism will first be dealt with, then the effects of insulin, adrenaline, and the combined effects of adrenaline and insulin. In Part II other disturbances of carbohydrate metabolism will be dealt with.

Variations in the pre-existing ratio of free to bound phosphate are extremely easily brought about by even slight alterations in the method of removing, cooling, mincing and fixing the muscle, so that unless, as shown by Embden and his school, the hydrolysis of the ester is prevented by the use of fluoride (which at the same time increases the synthetic power of the muscle), it may be difficult or impossible to distinguish changes which are due to the methods employed from those which have occurred *in vivo*. Special stress will therefore be laid upon the maximal synthesis *in vitro* produced by fluoride on skeletal muscle.

*Methods.* The methods employed in the preparation of the muscle specimens were based upon those used by Embden and others. The animals were bled, the muscles sprayed with ethyl chloride, carefully removed, and placed in a glass dish immersed in a freezing mixture. Liquid air was employed for freezing in some cases in order to determine the ratio of free to bound phosphate in the original fresh muscle, but the advantages of this method were more or less counteracted by certain mechanical difficulties in obtaining well-powdered specimens, so that throughout this paper the so-called A values have been obtained by the method first mentioned. One set of cooled and rapidly minced muscle specimens, in approximately 2 gm. quantities, was used for phosphate and lactic acid determinations, one half being employed for each determination. These specimens were placed as rapidly as possible in small cooled glass vessels, containing the various fluids to be mentioned afterwards. Another set of specimens, approximately 1 gm. each, were placed in centrifuge tubes of 30 c.c. capacity, containing a similar series of fluids and these specimens were used for glycogen determinations. The solutions used were the following.

To determine the pre-existing free phosphate value and the original content of lactic acid in the fresh muscle, one specimen (2 gm.) was placed in 10 c.c. 4 p.c. HCl saturated with NaCl, thoroughly mixed up, left for about one hour and the proteins then precipitated by mercuric chloride, filtered after 12 hours, mercury removed, and portions of the filtrate taken for phosphate determination (Embden's gravimetric

method (6) and for lactic acid estimation (Hirsch-Kaufmann). These specimens gave the so-called A values.

To determine the total amount of phosphate (free and bound), the so-called B values, approximately 2 gm. of the minced muscle were placed in 10 c.c. 2 p.c.  $\text{NaHCO}_3$ , kept for 2 hours at  $45^\circ$ , deproteinised, filtered and phosphate and lactic acid determined as described above. The maximal lactic acid production on warming in alkaline solution was thus also obtained. As regards the phosphate, from the B value of the total phosphate and the A value of the free,  $B - A$  gives the organically bound. Other specimens of approximately the same weight were placed in the following fluids (10 c.c. each) and kept at  $18^\circ$  for 4 hours, N/10 sodium fluoride in 2 p.c.  $\text{NaHCO}_3$  (C value), the same fluid with .4 p.c. glycogen (D value), the same fluid as C but with smaller (E) or larger (G) addition of phosphate, and the same fluid as D but with smaller or larger additions of phosphate (F and H respectively).

The specimens for glycogen determination were placed in 5 c.c. of the same fluids for the determination of the C to H values, the A value (glycogen content of fresh untreated muscle) was obtained from 1 gm. muscle placed in 5 c.c. 60 p.c. NaOH and boiled for 2 hours. The B value for glycogen was only determined in a few cases, because it was found that practically all the glycogen disappeared when minced muscle was kept in 2 p.c.  $\text{NaHCO}_3$  for 2 hours at  $45^\circ$ . The other specimens were placed in the same fluids as for phosphate and lactic acid determinations and kept for the same period in these solutions, then NaOH was added up to 60 p.c. and the mixture boiled as before. The glycogen was determined in each case as follows. After heating with the alkali, the solution was diluted with an equal quantity of water, the glycogen precipitated with an equal volume of 96 p.c. alcohol, centrifuged, deposit dissolved in water, reprecipitated with alcohol, and again centrifuged. The deposit was now dissolved in a small quantity of water, acidified (up to 2.5 p.c.) with HCl, hydrolysed by boiling for 2 hours, and the sugar determined by Bang's latest method. The blood sugar was determined by Folin's and J. A. Milroy's<sup>1</sup> method, and the liver glycogen was determined in most cases, using the method above described.

The following values were therefore obtainable if the series were a complete one.

1. The pre-existing free phosphate, lactic acid and glycogen (A).
2. The total phosphate (B) and therefore the bound phosphate ( $B - A$ ), and the maximal lactic acid production (B).

<sup>1</sup> In course of publication.

3 The amount of the pre-existing free phosphate synthesised by NaF (A - C), and by NaF glycogen (A - D), along with the accompanying lactic acid and glycogen changes

4. The amount of added phosphate which could be synthesised per gram muscle in the presence of added glycogen (F, H) or without addition of glycogen (E, G).

Before taking up a complete series such as has been outlined, it is advisable to refer to the distribution of the free and bound phosphate in the fresh muscles of normal animals in order to see whether a fairly constant ratio is met with in different animals of the same species, so that a standard might be used for comparison with abnormal cases. From our own analysis, the maximal, minimal and average values for A and B and the percentages of bound and free phosphate were as follows

	p c $H_2PO_4$		Average p c
	B	A	
1. Cat			
Maximal	531	330	
Minimal	417	302	Bound 34
Average	472	312	Free 60
2 Dog			
Maximal	457	280	
Minimal	370	233	Bound 39.7
Average	409	257	Free 60.3
3 Rabbit (mixed muscle)			
Maximal	623	373	
Minimal	540	301	Bound 41.8
Average	585	340	Free 58.2

Embden, Schmitz and Meincke(8) give as value for dog's muscle 37.4 p c bound, while Embden and Adler (1c) give, as the average for mixed rabbit's muscle, about 15 p c bound. It is evident from Embden's and our own figures that the maximal phosphate values, bound and free, are met with in the rabbit, and the minimal in the dog. Our own analyses show intermediate values for the cat. As regards the average percentages of bound, these are highest in the rabbit and lowest in the cat. As the values were found by us to be more constant in the cat than in the rabbit, partly due to the difficulty of obtaining muscle of exactly the same type in the latter, and the great differences exhibited by pale and red muscle, attention has been directed mainly to the former.

#### *Phosphate metabolism in normal muscle*

The behaviour of the normal cat's muscle under the action of fluoride, with and without the addition of the components used for esterification,

will be given in two cases. In each case the changes per gram of muscle are given.

No. 1. Normal. *The fresh muscle before the action of fluoride contained per gm.*

	I	II	III
	Inorganic $\text{H}_3\text{PO}_4$ mgm.	Lactic acid mgm.	Glycogen mgm.
A values	3.02	0.86	10.5

After warming in bicarbonate solution at  $45^\circ$  the values were

B values	4.96	3.66	—
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The glycogen may be taken as having practically disappeared.

The hydrolysis has set free 1.94 mgm.  $\text{H}_3\text{PO}_4$ , which were present in the fresh muscle in combined form. The ratio of the bound to the free phosphate was therefore 39.1 : 60.9.

The same muscle after the action of fluoride bicarbonate gave the following values per gm. muscle.

C values	0.36	0.49	1.95
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Of the total  $\text{H}_3\text{PO}_4$  per gm. muscle (4.96 mgm.) only 0.36 mgm. is now present in the free form, so that the ratio of bound to free is 93.8 : 6.2.

In order to determine the actual amount of the free phosphate which has been synthetised, we start with the A phosphate value, and it is evident that 1 gm. muscle has synthetised 2.66 mgm. free  $\text{H}_3\text{PO}_4$ , out of a total free  $\text{H}_3\text{PO}_4$  of 3.02 mgm. (88 p.c. synthesis). During this synthesis there is per gm. muscle a disappearance of 0.37 mgm. lactic acid and a consumption of 8.55 mgm. glycogen. If we take 0.82 mgm. glycogen as requisite for the synthesis of 1 mgm.  $\text{H}_3\text{PO}_4$  to form the hexosediphosphate, then for the synthesis of the 2.66 mgm. 2.18 mgm. glycogen only would have been required, so that apart from a possible synthesis of the lactic acid which has disappeared, the glycogen consumption is much greater than would have been required for the lactacidogen synthesis. It is to be noted however that lactic acid production has not only been checked by the fluoride but part has been used up.

*Action of fluoride bicarbonate solution to which 13.2 mgm. glycogen were added per gm. muscle.* The values before and after synthesis were:

	I	II	III	
Before synthesis	3.02	0.86	23.8	(10.5 originally present 13.3 added)
After synthesis	0.32	1.48	2.2	
(D values)				

Or

$\text{H}_3\text{PO}_4$		
Synthesis per gm. muscle	Lactic acid produced	Glycogen consumed
2.7 mgm.	0.62 mgm.	21.58 mgm.

The ratio of the bound to the free phosphate after synthesis was 94.7 : 5.3.

One gram muscle in the presence of fluoride, bicarbonate, glycogen has synthesised 2.7 mgm out of 3.02 (89.1 p.c.), a better synthesis than from the action of fluoride without the further addition of glycogen. At the same time 0.62 mgm lactic acid was produced, and 21.6 mgm. glycogen were used up. For the phosphate synthesis which took place only 2.2 mgm glycogen were required and, as only 0.62 mgm. lactic acid was produced, by far the larger part of the glycogen has been otherwise transformed, in all probability into hexoses which, as Embden has shown, do not improve the synthetic action of the fluoride.

*Synthesis of added phosphate* As in this case the synthetic action of the fluoride on the pre-existing phosphate has been so great, more phosphate with and without glycogen was added in order to determine how efficiently added phosphate could be synthesised.

1 Addition of phosphate alone

	I	II	III
Before synthesis	7.65	0.86	10.5
	(3.02 originally present 4.63 added)		
After synthesis (E values)	1.31	0.62	3.52

or 6.34 mgm  $H_3PO_4$  out of 7.65 have been synthesised (82.2 p.c.) and with this extremely good synthesis, 0.24 mgm lactic acid has disappeared and 6.98 mgm glycogen have been consumed, that is to say a smaller glycogen consumption than during the fluoride synthesis of the pre-existing phosphate and glycogen. There has been here also not only a check in lactic acid production but a small amount has disappeared. For the synthesis of the 6.34 mgm  $H_3PO_4$  approximately 5.2 mgm. glycogen would have been required, so that in this case by far the larger quantity of glycogen originally present was used for the additional synthesis. In addition some of the lactic acid which disappeared might have been used for the synthesis, but it is important to note that the esterification has occurred with so little waste of glycogen and without any lactic acid production.

*Addition of phosphate plus glycogen to the fluoride solution* The values before and after synthesis were

	I	II	III
Before	8.52	0.80	23.6
	(3.02 originally present 5.5 added)		
After (F values)	0.58	1.53	nil
	(10.5 originally present. 13.1 added)		

One gm. of muscle was therefore able to synthesise 7.94 mgm. out of 8.52 (93 p.c.) while of the added 5.5 mgm., 5.23 were synthesised

(95 p.c.). It is evident that the synthesis of this added phosphate has been as good as the synthesis of the pre-existing phosphate under the same conditions. During this synthesis all the glycogen disappeared and 0.97 mgm. lactic acid was produced per gm. muscle. For the amount of phosphate synthetised only about 6.5 mgm. glycogen would have been required so that there has been a great waste of glycogen. Only a small part of the glycogen has been transformed into lactic acid. In this case undoubtedly more phosphate could have been synthetised with the glycogen present, and, therefore, in the next specimen of normal muscle, the synthesis after larger additions will be studied.

No. 2. Normal cat's muscle.

The results will be condensed, the same values (A, B etc.) being referred to as in the preceding example.

	I	II	III
Per gram muscle	$H_3PO_4$	Lactic acid	Glycogen
A values	3.30	1.21	4.38
B values	5.31	6.65	nil

2.01 mgm.  $H_3PO_4$  have been set free during complete hydrolysis, and therefore in the fresh muscle 37.9 p.c. was in bound form and 62.1 p.c. free. The lactic acid which has been produced might have arisen from the hexosediphosphate (1.8) and from the glycogen (3.64), leaving a small amount of glycogen (if the analyses were correct) to be otherwise transformed. After fluoride action on the pre-existing constituents, the C values were:

I	II	III
0.67	0.93	1.15

The ratio of the combined to the free phosphate was now 87.4:12.6 and 2.63 mgm. of the original 3.3 mgm. of free phosphate have been synthetised (approximately 84 p.c.). This synthesis would require 2.15 mgm. glycogen and over 3 mgm. have been used up. At the same time, as in the preceding case, there has been a slight disappearance of lactic acid, viz. 0.28 mgm. It is evident that the glycogen which was consumed has been mainly used for esterification.

After the action of fluoride bicarbonate plus glycogen 17.28 mgm. (4.38 originally present 12.9 added) the D values were:

I	II	III
0.81	0.66	6.28

The ratio of the bound to the free phosphate after this action was 84.8:15.2, and 1 gm. muscle had synthetised 2.49 mgm. out of 3.30 (75.4 p.c.), slightly less than before the glycogen addition, so that for

improvement in esterification it was phosphate rather than glycogen which was required. Although so much glycogen had been consumed beyond that required for the formation of the ester, namely 11 mgm., and only 2.04 were required, none of the wasted glycogen went to form lactic acid, as there was an actual disappearance of 0.55 mgm. lactic acid per gm.

*Synthesis of added phosphate in the fluoride solution.* Larger amounts of phosphate were added than in the preceding case, one set with 9.4 mgm. addition, the other with slightly over 14 mgm. The  $H_3PO_4$  was added as usual in the form of  $Na_2HPO_4$ . The amount of phosphate present per gm. muscle was 12.7 mgm. (3.3 originally present plus 9.4 added).

	I	II	III
Before synthesis with fluoride	12.7	1.21	4.38
After (F values)	5.63	2.07	0.21

7.07 mgm.  $H_3PO_4$  were therefore synthesised out of a total 12.7 and this was accompanied by a consumption of 4.17 mgm. glycogen. If glycogen had been the sole source of the carbohydrate component of the synthesised ester, then 5.97 mgm. would have been required, therefore, if the analyses were correct, the hexose required must have in part been derived from some other source. That this source was not the lactic acid is evident from the increased formation of this acid. In this case it is clear that it is the glycogen which is lacking for optimal synthesis, and in the next specimen, glycogen was added along with a rather smaller addition of phosphate to obtain a suitable proportion of the components for esterification.

F values before and after synthesis.

	I	II	III
Before	10.90	1.21	19.48
(3.3 originally present: 7.0 added)			(4.38 originally present: 15.1 added)
After	1.34	0.77	5.38

9.56 mgm.  $H_3PO_4$  have been synthesised out of a total 10.9 (87.7 p.c.) so that the addition of the glycogen has greatly improved synthesis. For this synthesis 7.91 mgm. of glycogen would have been required so that much more glycogen has disappeared (11.1) than is required for the synthesis, and none of this has gone for lactic acid production because, with the improved esterification, the acid formation which was observed in the wasteful synthesis of F has been completely checked by the addition of the glycogen. It is evident that a larger quantity of phosphate could have been synthesised for the amount of glycogen

present, and that this is the case will be seen from the H values. Before giving those values, however, it will be of interest to note the effect produced by adding the larger quantity of phosphate without glycogen addition (G values):

	I	II	III
Before synthesis	17.5	1.21	4.38
	(3.3 originally present: 14.2 added)		
After synthesis	9.7	1.00	0.18

7.8 mgm.  $\text{H}_3\text{PO}_4$  have been synthesised, only 0.73 mgm. more than with the much smaller phosphate addition, so that evidently maximal synthesis had been practically attained with the smaller quantity until a further supply of glycogen was added. The glycogen of the muscle was practically exhausted, and it is of interest to note that the lactic acid production observed in F has been checked and some of it possibly used for the slightly improved synthesis. It is apparent however that, even if all the glycogen consumed and the lactic acid which disappeared had been used for synthesis, they would have been insufficient for the synthesis of 7.8 mgm.  $\text{H}_3\text{PO}_4$  as for this purpose approximately 6.4 mgm. of glycogen or its equivalent would have been required. It is evident therefore that a muscle can obtain the hexose for synthesis from some source other than these, although the most effective carbohydrate for the esterification process is undoubtedly the glycogen.

We shall now consider the H values, when with the larger amount of added phosphate there is also an addition of glycogen.

	I	II	III
Before synthesis	17.0	1.21	20.28
	(3.3 originally present: 13.7 added)		(4.39 originally present: 15.90 added)
After synthesis (H values)	2.9	1.21	6.68

14.1 mgm.  $\text{H}_3\text{PO}_4$  have now been synthesised out of a total 17 mgm. free phosphate (83 p.c.), and the esterification has been accompanied by a disappearance of 13.6 mgm. glycogen. 11.56 mgm. of glycogen would have been sufficient for the synthesis so that the amount consumed is only slightly greater than the quantity required for the ester formation. At the same time lactic acid production has just been checked. This may be regarded as an optimal type of synthesis. It is of interest to note that under fluoride the muscle has been able to esterify *in vitro* more than four times the quantity of free  $\text{H}_3\text{PO}_4$  originally present in the muscle and with an extreme economy both as regards glycogen consumption and lactic acid production.



It is evident from the preceding experiments that by the action of fluoride both the pre-existing and added inorganic phosphate can be very greatly reduced. What effect will fluoride have on hexosediphosphate added to muscle? An experiment was carried out on another specimen of normal cat's muscle.

Two portions of minced muscle (1.22 gm.) were taken and one portion placed in (a) 5 c.c. fluoride bicarbonate solution and the other in

(b) 5 c.c. fluoride bicarbonate solution containing 8.5 mgm. sodium fructose diphosphate.

Both mixtures were kept at 18° for two hours

(a) then contained 0.49 mgm. free  $H_3PO_4$

(b) " " 0.37 " " "

It is evident that not only has the fluoride prevented hydrolysis of the ester, but the fructose diphosphate has increased the synthesis of the pre-existing free phosphate. When a similar quantity of the fructose diphosphate was added to 1 gm. of muscle in 5 c.c. isotonic NaCl containing 2 p.c.  $NaHCO_3$  there was, after two hours at 15°, a 29 p.c. hydrolysis of the added ester, in addition to complete hydrolysis of the pre-existing lactacidogen.

#### *Phosphate metabolism in insulinised muscle*

The following may be taken as typical of the behaviour of the muscles of a cat under the influence of insulin, when examined in the same way as in the normal animals. The muscles of many animals (dog, rabbit and cat) under the influence of insulin were examined, but not with the same completeness as in the one to be described.

Ten units of insulin were injected and after 3½ hours the cat was killed and bled. The animal showed no signs of convulsions but was distinctly under the influence of insulin and the blood sugar was 0.8 p.c. Liver glycogen was 2.65 p.c. The fresh muscle before the action of the fluoride contained per gram, before warming (A), after warming (B):

	I	II	III
	Free $H_3PO_4$	Lactic acid	Glycogen
A values	3.20	2.06	8.48
B values	4.82	4.85	nil

The ratio of the bound to the free phosphate was therefore 33.6 : 66.4

After the action of fluoride

C values	0.38	practically none	4.96
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An extremely small amount, 0.38 out of the total 1.82 mgm.  $H_3PO_4$ , was now in the free form or the ratio of bound to free phosphate is 92.1 : 7.9. Taking the A and C values, 1 gm. muscle has synthesised 2.82 mgm. out of the original 3.20 mgm. free  $H_3PO_4$  (88.1 p.c.). Accom-

panying this synthesis there has been a disappearance approximately of 2 mgm. lactic acid along with a consumption of 3.52 mgm. glycogen. 2.31 mgm. glycogen would have furnished enough hexose for the esterification which had occurred, and the lactic acid which disappeared might have been used to furnish hexose for the synthesis of 1.85 mgm.  $\text{H}_3\text{PO}_4$ . Some of the lactic acid and glycogen which disappeared must therefore have been used in some other way. The muscle has reacted to the fluoride in much the same way as a good normal muscle, but there seemed to be a holding up of the glycogen in the insulin treated case, the C glycogen value being evidently higher than in the normal muscle, having originally a similar or higher glycogen content.

*The action of fluoride bicarbonate glycogen.* From a study of the preceding fluoride results obtained without further addition of glycogen, it was evident that sufficient of the polysaccharide remained at the end to synthesise more phosphate and hence it was not to be expected that the addition of more would improve synthesis. The following results show this to be the case, the A and D values being:

	I	II	III	
A (before synthesis)	3.20	2.06	20	(8.48 originally present: 11.52 added)
D (after synthesis)	0.54	1.49	9	

After the synthesis therefore 0.54 out of a total 4.82 mgm.  $\text{H}_3\text{PO}_4$  was free and the ratio, bound to free, 88.8 : 11.2, while 2.66 mgm. of the original 3.2 mgm. free  $\text{H}_3\text{PO}_4$  have been synthesised (83.1 p.c.). The synthesis has therefore not been improved by the addition of glycogen and the amount of lactic acid which has disappeared is less. The glycogen consumption is naturally greater with the added glycogen, but the consumption is less than with a corresponding addition to normal muscle.

*Synthesis of added phosphate under fluoride.* (1) With smaller quantity (5.3 mgm.  $\text{H}_3\text{PO}_4$  per gm. muscle):

	I	II	III
Before synthesis	8.50	2.06	8.48
	(3.2 5.3 added)		
After synthesis (F values)	1.75	1.57	1.73

The synthesis has been greatly improved by the addition of phosphate, 6.75 mgm. being removed out of a total 8.50 mgm. free  $\text{H}_3\text{PO}_4$  (79.4 p.c. synthesis), accompanied by a consumption of 6.75 mgm. glycogen and a disappearance of 0.49 mgm. lactic acid. About 5 mgm. glycogen would have been sufficient for the amount of phosphate synthesised, so that the major part of the glycogen has been used for esterification, and a small quantity of lactic acid has also disappeared.

(2) With larger quantity (8.6 mgm  $\text{H}_3\text{PO}_4$  per gm muscle)

	I	II	III
Before synthesis	11.80 (3.2 8.6 added)	2.06	8.48
After synthesis (G values)	4.27	1.03	1.73

A larger quantity of phosphate has been synthesised with the larger addition, viz. 7.53 mgm, while the amount of glycogen consumed has remained the same, but a larger quantity of lactic acid has disappeared. For this synthesis 6.17 mgm glycogen would have been required, and as the consumption was 6.75 mgm practically all the glycogen might have been used for the ester formation. It is probable however that the increased disappearance of lactic acid signifies that the improved synthesis is in part at least due to this acid being used for synthesis of the carbohydrate component.

*Synthesis of added phosphate along with the addition of glycogen under the action of the fluoride* (1) With smaller addition of phosphate (5.1 mgm  $\text{H}_3\text{PO}_4$  per gm muscle) along with 12.42 mgm glycogen addition

	I	II	III
Before synthesis	8.30 (3.2 5.1 added)	2.06	20.0 (8.48 12.42 added)
After synthesis (F values)	1.36	1.74	6.6

One gm. muscle has therefore synthesised 6.94 mgm  $\text{H}_3\text{PO}_4$  out of a total 8.3 (83.6 p.c.), accompanied by the consumption of 11.3 mgm glycogen and the disappearance of 0.32 mgm lactic acid. The glycogen addition has not greatly improved synthesis and as only 5.69 mgm. would have been required for the ester formed, a large proportion of the glycogen must have gone in some process other than esterification or lactic acid production.

(3) With larger addition of phosphate plus glycogen

	Phosphate addition per gm	10.40 mgm	
	Glycogen     "     "	10.92 mgm	
	I	II	III
Before synthesis	13.60	2.06	19.4
After synthesis (H values)	3.75	2.06	4.5

9.86 mgm  $\text{H}_3\text{PO}_4$  have been synthesised (73 p.c.), with no lactic acid production and a consumption of 11.9 mgm glycogen. For this synthesis 8.08 mgm glycogen would have been required. It is evident that for approximately the same glycogen consumption, the amount of phosphate synthesised is much greater with the larger than with the smaller phosphate addition (8.86 and 6.94 mgm per gm muscle respectively).

The synthetising power of the muscle in this insulinised animal was of much the same kind as that of a good normal muscle when tested under the same conditions. There has been some difference of opinion regarding the relative amounts of bound to free phosphate in the muscles of insulinised normal rabbits and in those of the untreated animals. Harrop and Benedict(9), Andova and Wagner(10), and Kay and Robinson(11) have found an increase in the hexosephosphate of muscle after insulin, while others, Soxhey and Allen(12), Collazo and others(13), and Eadie, Macleod and Noble(14) have usually found a slight decrease. The results evidently differ with the condition of the insulinised rabbits, whether well nourished or starving, with or without convulsions, etc. From a small number of experiments which we have carried out, the results appear to be variable, although higher than normal lactacidogen values may be met with in the insulinised animal. The muscles of three insulinised animals (10 units in each case) gave the following results:

	Blood sugar p.c.	Liver glycogen p.c.	H <sub>2</sub> PO <sub>4</sub>		Bound H <sub>3</sub> PO <sub>4</sub> p.c.	Remarks
			A p.c.	B p.c.		
1. Cat	·022	2·79	·300	·490	38·7	Convulsions
2. „	·080	2·65	·320	·480	33·6	No convulsions
3. Rabbit	·070	0·15	·286	·623	54	No convulsions

In one of the cats and in the rabbit (mixed muscle) the lactacidogen values were rather higher than in the average normal animal, while in the other cat it was about the same value. The glycogen and the lactic acid values (A, B and C have the usual significance) in the three specimens were:

	Lactic acid p.c.			Glycogen	
	A	B	C	A	C
1.	·221	·705	·055	1·35	·613
2.	·206	·485	—	0·848	·496
3.	—	—	—	0·235	·275

In both cats there was a high glycogen content in the muscles and also a high lactic acid percentage, the latter under fluoride practically disappearing. In the rabbit the glycogen content of the muscles was low and under the influence of fluoride there was no evidence of glycogen consumption. The liver glycogen was extremely low in the rabbit and about the average value in the cats.

#### *Phosphate metabolism in adrenalinised muscle.*

Two examples will be given. In one case (1) 1·5 mgm. adrenaline were injected and the animal killed and bled 3 hours later, in the other

two injections of 1.5 mgm were given with an interval of 1½ hours between the injections and 3 hours later the animal was killed (2)

In (1) the blood sugar was 0.36 p.c., in the other (2) it was 0.44 p.c. Liver glycogen (1) 2.045 p.c., liver glycogen (2) 0.337 p.c.

The fresh muscle in each case, contained per gm., before the action of fluoride

	I	II	III
A values	Free $H_3PO_4$ mgm	Lactic acid mgm	Glycogen mgm
(1)	3.15	0.30	1.55
(2)	3.85	0.43	4.98

After warming in bicarbonate solution at 45° C for two hours the B values were

(1)	1.75	3.00	0
(2)	5.35	4.90	0

The hydrolysis has set free 1.6 mgm  $H_3PO_4$  in (1) and 1.5 mgm in (2), per gm muscle, which had been present originally in the combined form. The ratio, therefore, of bound to free  $H_3PO_4$  is in (1) 33.68 : 66.32 and in (2) 28.72, that is to say the pre-existing hexosephosphate value was rather lower in each case than in the average normal muscle.

*Action of fluoride bicarbonate* This may be given briefly in the following form

The ratio of bound to free  $H_3PO_4$  in (1) was 61.1 : 35.6, and in (2) was 70 : 30. In (1) 1 gm of muscle synthesised 1.15 mgm  $H_3PO_4$  out of an original 3.15 mgm inorganic  $H_3PO_4$ , that is a 16 p.c. synthesis. In (2) 1 gm of muscle synthesised 2.24 mgm  $H_3PO_4$  out of an original 3.85 mgm (58 p.c.). It is evident that the synthetic action of the fluoride on the muscle phosphate in adrenalinised cases is much less than in the normal ones. During this synthesis there is per gm muscle a production of 1.08 mgm lactic acid in (1) and of 0.22 mgm lactic acid in (2) along with the disappearance of 0.75 mgm glycogen in (1) and 1.11 mgm in (2). Slightly more than 1 mgm glycogen would be required to supply the necessary hexose for the amount of ester formed during the fluoride synthesis in (1) and nearly 2 mgm would be required for the synthesis in (2).

Case (1) therefore differs from (2) in having insufficient glycogen for the sole supply of the ester formed, while in (2) there is sufficient. It is also to be observed that in (1) there is a greater lactic acid production than in (2). The better synthesis in (2) is therefore associated with a better glycogen supply and a smaller lactic acid production.

*Action of fluoride bicarbonate with addition of glycogen* In (1) 1 gm muscle after fluoride action synthesised 1.15 mgm  $H_3PO_4$  out of 3.15 mgm or 36.5 p.c. of the available inorganic  $H_3PO_4$ , and during

this synthesis 4.85 mgm. glycogen were used up, out of a total 15.45 mgm. of which 1.55 mgm. were present originally and 13.9 mgm. added, while 1.15 mgm. lactic acid were produced. After this action, the ratio of bound to free  $\text{H}_3\text{PO}_4$  was 57.9 : 42.1. For the synthesis of 1.15 mgm.  $\text{H}_3\text{PO}_4$  rather less than 1 mgm. of glycogen would be required and 4.85 mgm. glycogen have been used up without any improvement in synthesis. There has been a distinct increase in the lactic acid production, but by far the larger quantity of the glycogen has been otherwise transformed. The synthesis in this muscle both with fluoride alone and with fluoride glycogen is much worse than in the normal animal.

In the adrena1ised cat (2) 1 gm. of muscle synthetised 2.68 mgm.  $\text{H}_3\text{PO}_4$  out of 3.85 mgm. or 69.6 p.c. of the available inorganic  $\text{H}_3\text{PO}_4$ , and during this synthesis .9.8 mgm. glycogen disappeared out of 23.18 mgm. of which 4.98 mgm. were present originally and 18.2 mgm. added. For the synthesis effected, only 2.2 mgm. glycogen would have been required. The lactic acid production is very small, 0.53 mgm. Synthesis has been improved in this case by the addition of glycogen and lactic acid production has been checked. The ratio of bound to free  $\text{H}_3\text{PO}_4$  at the end of this synthesis was 78.14 : 21.86. Although the synthesis is not so good as in the normal cat's muscle it is distinctly better than in the former adrena1ised cat.

*Synthesis of added phosphate by fluoride alone.* In (1) as the synthesis of the original phosphate by fluoride was much poorer than in (2) fortunately a smaller quantity of phosphate was added. In this case (1) 3.65 mgm. inorganic  $\text{H}_3\text{PO}_4$  were synthetised out of 6.8 mgm. of which 3.15 mgm. were originally present and 3.70 mgm. added (53.2 p.c.). During the synthesis only 0.28 mgm. of the original 1.55 mgm. glycogen was used, and there was a production of 1.02 mgm. lactic acid. For the synthesis of 3.65 mgm.  $\text{H}_3\text{PO}_4$  almost 3 mgm. glycogen would be required, therefore for the formation of the ester, hexose must have been obtained from a source other than the glycogen.

In (2) 4.90 mgm.  $\text{H}_3\text{PO}_4$  were synthetised out of 12.45 mgm. of which 3.85 mgm. were present originally and 8.60 mgm. added (39.3 p.c.). The glycogen consumption accompanying this synthesis was doubtful, but, as in (1), there was a production of lactic acid (0.9 mgm.) per gm. muscle.

On testing (2) with a larger addition of phosphate, out of a total of 20.9 mgm.  $\text{H}_3\text{PO}_4$  per gm. (3.85 originally present plus 17.05 mgm. added) 1 gm. of muscle synthetised only 3.5 mgm.  $\text{H}_3\text{PO}_4$  (17.7 p.c.). During the synthesis 4.22 mgm. of glycogen were used up. For this synthesis

only 2.87 mgm. would be required. There was an increase in lactic acid formation of 0.48 mgm. Therefore in this muscle further addition of phosphate does not improve the synthesis but appears to harm it.

*Synthesis of added phosphate in fluoride solution containing glycogen.* In (1) 1 gm. muscle synthesised 2.3 mgm.  $H_3PO_4$  out of 6.85 mgm., of which 3.15 were originally present and 3.7 mgm. were added (33.5 p.c.), but this low percentage is due to the poor synthetic action of the fluoride on the pre-existing phosphate. If the fluoride acted to the same extent in this case, on the pre-existing phosphate, 68 p.c. of the added was synthesised. During this synthesis of 2.3 mgm.  $H_3PO_4$ , 5.5 mgm. glycogen out of 16 mgm. present, were used up and only 1.88 mgm. would be required, while 0.53 mgm. of lactic acid was produced.

In (2) 7.38 mgm.  $H_3PO_4$  out of 12.25 mgm. (3.85 mgm. originally present plus 8.4 mgm. added) were synthesised per gm. (60 p.c.). The synthesis was accompanied by a disappearance of 9.34 mgm. of glycogen out of 19.3 mgm. present. The lactic acid value was not obtained. About 6 mgm. glycogen would be required for this synthesis, so that the larger portion of the glycogen consumed has been used for synthesis. On testing muscle (2) with a larger quantity of added phosphate it was able in the presence of excess glycogen (21 mgm.) to synthesise only 8.43 mgm.  $H_3PO_4$  per gm. out of 23.45 mgm. (3.85 mgm. originally present plus 19.6 mgm. added) (29 p.c.). The lactic acid production during this synthesis was 0.37 mgm.

It is evident that the muscle from adrenalectomised cats is unable to synthesise both the pre-existing and the added phosphate to the same extent as the normal muscle under the action of fluoride.

#### *Phosphate metabolism in insulinised and adrenalectomised muscle.*

Two examples will be given where the combined effects of insulin and adrenaline, on cat's muscle, were studied.

No. 1. The cat was given an insulin injection of 10 units. In two hours it developed convulsions and adrenaline was injected, 1.5 mgm. when the convulsions began, and another 1.5 mgm. after about half-an-hour. Two hours after the first adrenaline injection the animal had completely recovered, and the muscle was then taken for examination. The blood sugar was 0.21 p.c.

No. 2. 3 mgm. adrenaline were injected (first 2 mgm. and after 1 hour 1 mgm.) and 2½ hours after the first injection 10 units of insulin were injected and a further injection of 20 units insulin one hour later. Two hours after the first insulin injection the animal was killed and

bled. There were no signs of convulsions. The blood sugar was .091 p.c.

The fresh muscle in each case contained per gm., *before the action of fluoride*,

	I	II	III
	Free $H_3PO_4$	Lactic acid	Glycogen
	mgm.	mgm.	mgm.
A values (1)	3.20	1.13	4.60
(2)	2.88	0.65	4.52

After warming in bicarbonate solution at 45° C. for two hours

B values (1)	5.40	3.22	0
(2)	4.88	4.84	0

From the values obtained after hydrolysis the ratio of bound to free  $H_3PO_4$  was in (1) 40.7 : 59.3, and in (2) 40.98 : 59.02. The organic phosphate was here rather higher even than in normal muscle, and distinctly higher than in adrenalised animals.

*After the action of fluoride bicarbonate* it was found that the following changes had been produced per gm. muscle.

In (1)	2.88 mgm. synthesised	0.19 mgm. disappeared	1.42 mgm. consumed
(2)	1.96 " "	No change	1.82 " "

The ratio of bound to free  $H_3PO_4$  was in (1) 94.26 : 5.74, and in (2) 81.2 : 18.8.

In (1) 2.88 mgm. out of a total 3.2 mgm. free  $H_3PO_4$  were synthesised (80 p.c.), and in (2) 1.96 out of a total 2.88 (68 p.c.). Even in (2) the synthesis was much better than the most favourable one in the untreated adrenalised cat, while in (1) the synthesis was as good as in the best normal muscle. In (1) during the synthesis, there was a consumption of only 1.42 mgm. glycogen, while for the synthesis of the free phosphate which disappeared 2.32 mgm. would have been required. Although the muscle could have supplied this amount, as at the end of the synthesis it still contained 3.18 mgm. glycogen, the hexose used for synthesis must have been derived from some other source. There was an extremely small lactic acid production.

In (2) during the synthesis 1.82 mgm. glycogen were consumed, and 1.60 mgm. would have been sufficient for the amount of the ester formed. The glycogen consumption is less than the normal muscle or in the muscle of adrenalised cats. During the process the lactic acid remained unchanged.

*Action of fluoride, bicarbonate, glycogen.* Glycogen addition 11.9 mgm. in (1), 13.08 in (2) per gm. muscle.

In (1)	1.39 mgm. $H_3PO_4$ were synthesised from 3.2 mgm. (43.4 p.c.)
(2)	2.10 " " " 2.88 mgm. (72.9 p.c.)

In (1) the synthesis was effected with the consumption of 5.56 mgm. glycogen, and the production of 0.28 mgm. lactic acid. In (2) the synthesis was accompanied by the consumption of 11.3 mgm. glycogen and



the formation of 1.19 mgm lactic acid. The glycogen consumption was much more in both cases than was required for the esterification. The ratio of bound to free phosphate after the synthesis was in (1) 66.3 : 33.7 and in (2) 85.6 : 14.4.

*Synthesis of added phosphate in fluoride alone* (a) With a small addition in (1) 2.6 mgm or 5.8 total, and in (2) 8.5 mgm or 11.38 total

In (1) 5.2 mgm out of the 5.8 were synthesised (90 p.c.) and in (2) 5.09 out of 11.38 accompanied by in (1) a glycogen consumption of 3.04 mgm and in (2) 3.48 mgm along with a production in (1) of 0.51 mgm lactic acid and in (2) of 1.04 mgm. In (1) it appears as if the component lacking for synthesis were phosphate, because addition of glycogen alone did not improve it, while a further supply of phosphate without glycogen resulted in extremely good synthesis. On the other hand in (2) a rather smaller quantity of phosphate was synthesised although the supply was much greater, while with added glycogen without phosphate addition the synthesis was much better than in (1). The lactic acid production was also less in (1) than (2). Evidently the component lacking in (1) was the phosphate and in (2) the glycogen.

(b) With a larger phosphate addition to (2) (without addition of glycogen) namely 17.6 or a total of 20.4 mgm  $H_3PO_4$ , 1 gm muscle synthesised 8.3 mgm with a consumption of 3.78 mgm glycogen and a production of 1.01 mgm lactic acid, so that even in (2) a larger addition of phosphate improved synthesis with only slightly greater glycogen consumption and lessened lactic acid formation, both changes being characteristic of good synthesis.

*Synthesis of added phosphate plus glycogen* In (1) phosphate addition 2.49, total 5.67, and glycogen addition 11.5 mgm per gm muscle. In (2) phosphate addition 7.7, total 10.58, glycogen addition 15.28

In (1) 1 gm. muscle synthesised 5.3 mgm out of 5.67

(2) " " 6.66 " 10.58

along with in (1) a 5.3 mgm glycogen consumption and 1.3 mgm lactic acid production and in (2) 12 mgm and 0.65 mgm. respectively

It was evident that in (1) the maximal synthesis for the phosphate present had been attained. Unfortunately this muscle was not tested with a larger supply of phosphate. In (2) the synthesis was tested with a larger phosphate supply along with 20 mgm glycogen.

It was found that, out of a total 22.18 mgm  $H_3PO_4$ , 10.5 mgm were synthesised along with a consumption of 18.5 glycogen and a production of 2 mgm lactic acid. This synthesis, although much better than the muscles of the untreated adrenalectomised cat could have accomplished, was

not so good as the esterification in the normal or insulin cases. The amount of glycogen required for the synthesis of 10.5 mgm.  $\text{H}_3\text{PO}_4$  would be 8.61 mgm. and 18.5 mgm. were actually consumed along with a production of 2 mgm. lactic acid per gm. muscle. That this glycogen waste has not been due to lack of free phosphate for synthesis is evident from the fact that 53 p.c. of the total phosphate was still in the free form at the end of the synthesis. Probably the excess glycogen was transformed into hexose, and in this form could not improve the synthetic action of the fluoride as Embden and Euler (*l.c.*) have shown. The muscles of the insulin treated adrenalectomised cat, as regards their synthetic power in the presence of fluoride, occupy an intermediate position between the normal or insulin and the adrenalectomised cases.

#### DISCUSSION.

Under the influence of fluoride, the *normal muscle* could synthesise 80-90 p.c. of the pre-existing free phosphate with the glycogen present. This synthesis was always accompanied by a greater consumption of glycogen than was required for esterification, but the lactic acid production was always checked or there was an actual disappearance of the acid. In the presence of added glycogen, if the pre-existing store were a good one, there might be a very slight rise in lactic acid production, but this was usually checked or some might even disappear. The muscle might under these conditions consume nearly three times as much glycogen as it would do, were the sole supply the pre-existing store, and yet there might be no improvement in synthesis or only a slight one. The beneficial effect of glycogen addition was more evident after the addition of more phosphate. The amount then synthesised depended upon the proportions of phosphate and glycogen present. In cases where a large proportion of the added phosphate disappeared and there still remained a fair amount of unaltered glycogen, then the normal muscle was still able to synthesise more phosphate without further addition of the carbohydrate component of the ester. The optimal type was an approximate 80 p.c. synthesis for a certain addition of phosphate along with a glycogen consumption only slightly more than would be required for the synthesis, and, at the same time, a check in lactic acid formation or an actual disappearance of a portion of the acid.

For optimal types of synthesis see Table I (Cat (1) E and Cat (2) H). The two specimens differ in this respect that in (1) the original glycogen content of the muscle was high, and a good synthesis could be produced by addition of phosphate alone, while in (2) the glycogen content was

low, and, before a good synthesis could be obtained, glycogen as well as phosphate required to be added. When phosphate was added to a muscle with low glycogen content, synthesis might occur, the process then requiring more hexose than could have been furnished by the quantity of glycogen consumed, and in such a case there was a production of lactic acid. Apparently the synthesis under these conditions required a preliminary lactic acid production, some of which might be synthetised. The muscle of the *insulinised* cat behaved in much the same way as normal muscle, under the action of fluoride, both as regards the synthesis of the intrinsic and the added phosphate. It also showed the same check in production, or even disappearance, of lactic acid, but there seemed to be a tendency for the insulinised muscle to hold up glycogen, especially the pre-existing store, although there was also evidence of a sparing action on added glycogen. In the muscle of the *adrenalised* cat the synthesis under the influence of fluoride was greatly diminished, that of the pre-existing phosphate varying from 40-60 p.c. A lactic acid production always accompanied the synthesis even when the glycogen which disappeared was sufficient to account for the synthesis. After glycogen addition the synthesis was only slightly improved or might be actually diminished and, in the latter case, synthesis of added phosphate also could not be improved by the addition of glycogen, of which only a comparatively small quantity was used. When the added glycogen did improve synthesis, the further addition of phosphate gave rise to a better esterification but this was never so good as with normal muscle, and the maximal amount of added phosphate which could be synthetised without glycogen addition was markedly less than in the normal. In all the synthetic processes lactic acid appeared. As regards the actual condition of the adrenalised animal's muscles at the outset, the glycogen content was always low and the lactic acid percentage much smaller than in the normal or insulin cases.

The muscle of the *insulin treated adrenalised* animals, compared with the untreated adrenalised, showed a marked improvement in the synthesis of both the intrinsic and the added phosphate, although it was seldom so good as in the normal or insulin normal cases. The synthetic processes were, as in the adrenalised cases, usually accompanied by an increase in lactic acid, while, in common with the normal insulin muscle, there appeared to be a sparing of the glycogen. Addition of glycogen, as in the adrenalin cases, only slightly improved or might even harm synthesis.

Tables I and II give in condensed form the synthesis of free phosphate and the accompanying changes in lactic acid and glycogen in normal and abnormal muscle respectively. Values are given in milligrams per gram muscle.

Column I gives  $H_3PO_4$  synthesis out of the total free  $H_3PO_4$ .

„ II „ the lactic acid changes, (–) = disappearance, (+) = production.

„ III „ the glycogen consumption.

Letters A to H have the significance referred to in the paper.

TABLE I.

	C			D			E		
	I	II	III	I	II	III	I	II	III
Cat 1	2.66 of 3.02 (88.1 %)	(–) 0.37	8.55 of 10.5	2.70 of 3.02 (89.4 %)	(+) 0.62	21.58 of 23.8	6.34 of 7.56 (83.8 %)	(–) 0.24	6.68 of 10.5
Cat 2	2.63 of 3.30 (79.7 %)	(–) 0.28	3.23 of 4.38	2.49 of 3.30 (75.4 %)	(–) 0.55	11 of 17.28	7.07 of 12.70 (55.6 %)	(+) 0.86	4.17 of 4.38

	F			G			H		
	I	II	III	I	II	III	I	II	III
Cat 1	7.93 of 8.7 (91.1 %)	(–) 0.97	23.6 of 23.6	—	—	—	—	—	—
Cat 2	9.56 of 10.9 (87.7 %)	(–) 0.44	14.1 of 19.48	7.8 of 17.5 (44.5 %)	(–) 0.21	4.2 of 4.38	14.1 of 17 (82.9 %)	No change	13.6 of 20.28

TABLE II.

	C			D		
	I	II	III	I	II	III
Adrenalin Cat 2	2.24 of 3.85 (58 %)	(+) 0.23	4.11 of 4.98	2.68 of 3.85 (69.6 %)	(+) 0.53	9.85 of 23.18
Insulin Adrenalin Cat 2	1.96 of 2.88 (68 %)	No change	1.82 of 4.52	2.10 of 2.88 (72.9 %)	(+) 1.19	11.3 of 17.6
Insulin Cat	2.82 of 3.20 (88 %)	(–) 2.0	3.52 of 8.48	2.66 of 3.20 (83.1 %)	(–) 0.57	11.6 of 20

	E			F		
	I	II	III	I	II	III
Adrenalin Cat 2	4.9 of 12.45 (39.3 %)	(+) 0.90	—	7.38 of 12.25 (60.2 %)	—	9.43 of 19.3
Insulin Adrenalin Cat 2	5.09 of 11.38 (44.7 %)	(+) 1.04	3.84 of 4.52	6.66 of 10.5 (63.4 %)	(+) 0.65	12 of 19.8
Insulin Cat	7.53 of 11.8 (63.8 %)	(–) 1.00	6.75 of 8.48	9.86 of 13.6 (72.4 %)	No change	14.9 of 19.4

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## EFFECT OF THYROID FEEDING ON SUGAR TOLERANCE. BY H. P. MARKS.

*(From the National Institute for Medical Research, London.)*

IN a recent communication from this laboratory<sup>(1)</sup>, evidence was presented in support of the view that one of the effects of increasing the amount of thyroid hormone in the body is to render the liver more sensitive to such stimuli as promote glycogenolysis and consequent discharge of sugar into the blood stream, while, conversely, elimination of the hormone by thyroidectomy diminishes the readiness with which the liver converts its glycogen into sugar. We were there concerned exclusively with this function of the liver, and did not consider the inverse one of conversion of sugar into glycogen. Various workers have suggested that sugar tolerance, *i.e.* the ability of an animal to dispose of ingested (or injected) sugar, is determined by the power of the liver to remove excess of sugar from the circulation, and convert it into glycogen. It is now generally recognised, however, that the liver is not the only organ involved, and that the pancreas plays an essential part.

On the relation of the thyroid hormone to sugar tolerance, the evidence of previous workers is conflicting. Thus, Cramer and Krause<sup>(2)</sup> state that, in dogs, sugar tolerance is slightly lowered by thyroid feeding, and Holm and Bornstein<sup>(3)</sup>, that the glycosuria following an injection of sugar is less after thyroidectomy. Underhill<sup>(4)</sup>, on the other hand, reports a diminished glucose tolerance in dogs after complete thyro-parathyroidectomy, while Kurijama<sup>(5)</sup> fails to find any alteration in the sugar tolerance of rats subjected to thyroid feeding. A large amount of clinical evidence of a similarly conflicting nature has also been put on record. Here it will suffice to mention the following case cited by Maclean<sup>(6)</sup>; it is of interest as affording an approach to the experimental conditions obtaining in our own investigations on thyroid-fed rabbits. A patient, suspected of being a diabetic on account of the character of his sugar tolerance curve, was subsequently found to be taking a course of thyroid tablets by mouth. After discontinuance of this treatment for some weeks, a repetition of the sugar tolerance test gave a curve of the usual, non-diabetic type.

In view of the unsatisfactory nature of the available evidence, a more detailed investigation of the changes in sugar tolerance of rabbits, during a period of thyroid feeding, was undertaken, and the results obtained appear to throw new light on the mechanism of blood-sugar regulation.

*Effect of thyroid feeding on response to glucose administration.*

In Fig. 1 are shown, for comparison, two curves depicting the course of the blood-sugar in a rabbit, following the injection into the ear-vein of one-eighth of a gram of glucose per kilo. of body weight. Curvo A

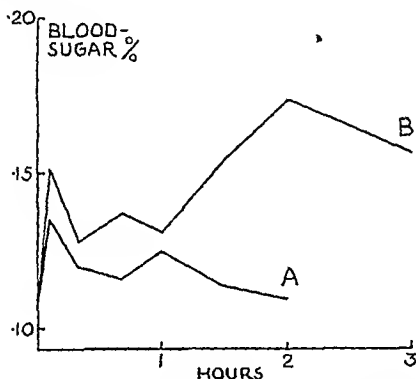


Fig. 1. Blood-sugar changes following an intravenous injection of glucose into a rabbit (A) when normal, (B) after 20 days' thyroid feeding. Note the late hyperglycemia in Curvo B.

shows the result obtained before thyroid feeding, Curve B that obtained after a period of 20 days' thyroid feeding. A comparison of the curves during the first hour following the glucose administration affords no evidence of such inability to dispose of excess of sugar as is seen in the diabetic organism. The injected glucose would appear to be removed from circulation equally rapidly in the normal and in the thyroid-fed animal. The point of particular interest, is that, in the thyroid-fed animal, the return of the blood-sugar to normal limits is followed by a secondary, pronounced hyperglycemia, far exceeding in magnitude and duration that directly produced by the injection of the sugar.

Had the blood-sugar changes immediately after the sugar injection

not been closely followed, the return to the normal level might have been missed, and the curve taken to indicate a diminished power of the organism to dispose of the injected sugar. Actually, we see such a conclusion to be erroneous; the late rise in the blood-sugar may probably be ascribed to a discharge of sugar from the liver, rendered, by thyroid feeding, hypersensitive to the action of some glycogenolytic stimulus, occurring as a sequel to the administration and normal disposal of sugar.

This late rise in the blood-sugar may also follow the administration of sugar by mouth, or subcutaneously; but it has not been observed in all our experiments on thyroid-fed rabbits, and would appear to come into prominence only in the later stages of thyroid feeding. A summary of the experiments in which this phenomenon has been observed is given below:

No. of days thyroid feeding	Amount of glucose per kilo. and method of administration	Maximum value % of blood-sugar during late rise	Time in hours after the administration of glucose at which this maximum occurred
20	.125 gm. intrav.	.157	2
20	.125 " "	.174	2
14	.125 " "	.133	5
23	.125 " "	.131	2
29	.125 " "	.133	2
26	.125 " subcut.	.152	3½
26	.125 " "	.145	2
23	1 " "	.224	3
27	1 " by mouth	.152	5-7
35	1 " "	.133 and .132	4-7
6	1 " "	.123	4
37	1 " "	.126	6
19	1 " "	.126	3

Since it has been shown that continued thyroid feeding ultimately results in the complete exhaustion of the glycogen reserves of the body<sup>(2)</sup>, the late rise in the blood-sugar should not be observed in the extremely advanced stages of thyroid feeding, if the explanation put forward above is true. Our experiments show, not only that this is the case, but, further, that the secondary hyperglycæmia is then replaced by a hypoglycæmia. Curve A in Fig. 2 illustrates the effect of giving  $\frac{1}{3}$  gram glucose per kilo. to a rabbit, after 20 days of thyroid feeding. It is seen that the slight secondary rise at 1½ hours was cut short by a progressive fall in the blood-sugar, ending in hypoglycæmic collapse, from which recovery was only effected by the injection of 1 gm. of sugar, and the giving of food as soon as this had produced its effect. The change produced by sugar injection in this rabbit was so surprising that the experiment was repeated on the following day, with the result indicated in Curve B, Fig. 2. It is seen that for over 3 hours, in the absence



of any injection, the blood-sugar did not fall. 0.6 gm. of glucose was then injected by the ear-vein. After a preliminary rise to over .2 p.c.,

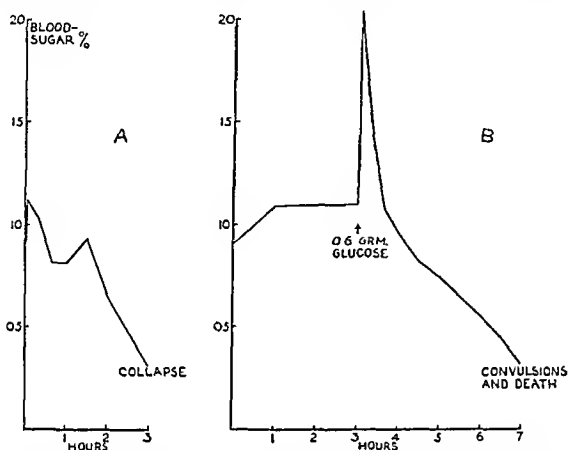


Fig 2 Hypoglycemia produced by injection of glucose into a thyroid fed rabbit. Note in A, the transient rise in blood sugar, and in B, the constancy of the blood sugar prior to the injection of glucose

the blood-sugar rapidly fell, in 2 hours it was down to .075 p.c., and after 4 hours the rabbit died after a few convulsive kicks, with a blood-sugar of .032 p.c. An examination of the liver showed it to contain no detectable amount of glycogen.

This phenomenon has also been observed to follow the administration of glucose by mouth. Indeed, the apparently spontaneous hypoglycemia which has invariably terminated our experiments on thyroid feeding, in the absence of other treatment, is presumably the result of the ingestion of carbohydrate food. This final stage is usually reached in from three to four weeks, when the rabbit receives the equivalent of 1.3 gm. dried thyroid gland per day, but occasionally a rabbit will survive for six or eight weeks.

#### *Effect of thyroid feeding on the response to small doses of insulin.*

A study of the response of rabbits to small doses of insulin at different stages of thyroid feeding affords an interesting parallel to these blood-

sugar changes following an injection of glucose. Thus Curve A, in Fig. 3, illustrates the production of a late rise of blood-sugar, similar to that already described as following glucose administration. In this case, a dose of  $\frac{1}{20}$  unit of insulin per kilo. was injected intravenously

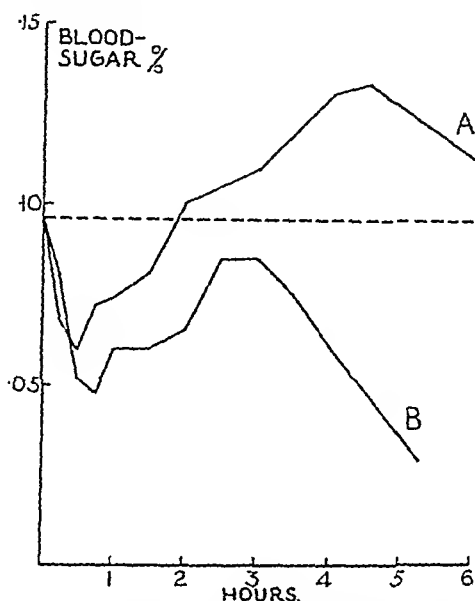


Fig. 3. Late hyperglycemia produced by injecting a small dose of insulin into a thyroid-fed rabbit. In (B) the late rise is insufficient to compensate the hypoglycemic effect of the insulin.

into a rabbit on the 23rd day of thyroid feeding. The late hyperglycemia observed in this case would appear to represent the discharge of the last trace of glycogen from the liver, for, on the following day, an injection of glucose gave rise to a rapidly progressive hypoglycemia, as a result of which the animal died in convulsions 2 hours after the glucose injection.

In the experiment illustrated by Curve B, the remaining glycogen reserve was apparently insufficient to restore the blood-sugar to the normal level, the slight rise in blood-sugar at the third hour being soon replaced by a second hypoglycemia, ending in convulsions and death,  $5\frac{1}{4}$  hours after the injection of the insulin. Here, again, no glycogen could be detected in the liver.

#### *Response to adrenalin.*

It has been stated (7) that the injection of adrenalin into animals previously rendered glycogen-free will still give rise to glycosuria, and

even cause the deposition of glycogen in the liver. According to Geolmuyden(8), this indicates that the sugar plethora following injection of adrenalin has some other source, besides the glycogen reserve in the liver, namely, the increased conversion of fat into sugar. Laufferberger(9) also suggests that insulin opposes this action, by stopping the conversion of fat into carbohydrate.

On the basis of this theory, it might be expected that an injection of adrenalin would arrest the hypoglycæmia following injection of glucose into a glycogen-free, thyroid-fed rabbit, by rendering sugar available from other sources. We find, however, that adrenalin has no effect on the course of the hypoglycæmia. In fact, the injection of .5 mgm. adrenalin into a rabbit in the advanced stage of thyroid feeding usually by itself brings on a fatal hypoglycæmia in a short time. Whether or not this is preceded by a detectable hyperglycæmia depends on the completeness with which the glycogen reserves have been exhausted.

#### *Discussion*

Many observers have drawn attention to the fact that, even in normal individuals, the rise in blood-sugar after the ingestion of sugar is frequently followed by a fall to slightly below the normal level. Folin(10), indeed, considers that this secondary hypoglycæmia is as regular a consequence of sugar ingestion as is the primary hyperglycæmia, and attributes it to the lessened need for sugar transport, as a consequence of the tissues having absorbed the ingested glucose, and thus become amply supplied with nourishment. He based this view on the observation that the ingestion of olive oil and of gelatin also give rise to a hypoglycæmia. Maclean(11), on the other hand, suggested that the liver was the organ chiefly concerned with the absorption and storage of the ingested sugar, and that this absorption might continue after the blood-sugar had been reduced to the normal level. More recently, Scvringhaus and Smith(12), among others, have advanced the suggestion that the blood-sugar is reduced to the normal level through the agency of insulin discharged from the pancreas, a hypoglycæmia ensuing if this discharge of insulin is more than sufficient to deal with the sugar ingested. In these circumstances, they suppose, the liver then again adjusts the balance by a discharge of sugar into the blood. The results reported in the present paper are in complete harmony with this conception.

Let us consider first the blood-sugar changes following a small dose of insulin. In the normal animal, the return of the blood-sugar to the

normal level after the initial hypoglycæmia is due, according to Macleod, to a compensatory discharge of glycogen from the liver. According to Burn and Marks(1) thyroid feeding renders the liver over-responsive to such stimuli (e.g. adrenalin) as promote a discharge of sugar into the blood. The secondary hyperglycæmia observed in the thyroid-fed rabbit after insulin may therefore be attributed to an over-compensation by the liver, carrying the blood-sugar above the initial level. As the exhaustion of the glycogen reserves proceeds, this over-activity of the liver, although still present, is seen to become less and less effective, until finally, when the liver has no more glycogen to discharge, the hypoglycæmia produced by even a small dose of insulin proceeds unchecked to a fatal termination.

Turning now to the parallel changes observed in the behaviour of the blood-sugar after an injection of glucose, the disappearance of the excess of glucose is to be ascribed, according to our theory, to a stimulation of the pancreas to secrete insulin. Such an assumption receives direct support from the experiments of Spiro and Staub(13). In the normal animal, any excessive action of the liberated insulin is compensated by a discharge of sugar from the liver, so that the normal blood-sugar level is restored. In the thyroid-fed animal, however, the over-responsiveness of the liver again comes into play, and the excessive discharge of sugar may be sufficient to cause a marked secondary hyperglycæmia. If every carbohydrate meal is followed by such an excessive breakdown of glycogen, it is easy to see how the glycogen reserves may in time become completely exhausted. The effect of the progressive depletion of the glycogen stores may be followed here, just as in the case of insulin injections. The effectiveness of the compensatory function of the liver is seen to diminish progressively, until finally only the insulin effect remains. Then the insulin, secreted in response to the entry of sugar into the blood-stream, removes from the circulation not only the excess of sugar, but, in addition, the whole of the small remnant of available glucose in the body, so that the fall in the blood-sugar continues, unchecked by any compensatory discharge of sugar from the liver, until it is terminated by the death of the animal in hypoglycæmic convulsions.

A similar observation on the clinical side has recently been reported by Weil and Laudat(14). These workers describe a case of renal glycosuria, in which the ingestion of 30 gm. glucose was followed by a fall in the blood-sugar to  $\cdot 048$  p.c., accompanied by weakness and a sense of hunger, but none of the other symptoms of hypoglycæmia.

Here, also, we may expect that the glycogen reserves of the patient are depleted, and the hypoglycæmia is presumably due to the uncontrolled action of insulin, discharged from the pancreas under the stimulus of the ingested sugar.

It was thought that the phenomenon might be observed, even in the presence of ample glycogen reserves, if these reserves could be rendered inaccessible by paralysing the sympathetic endings by means of ergotamine. Under such conditions, the hyperglycæmia caused by an injection of glucose was followed by a fall in the blood-sugar to 07 p.c., but no hypoglycæmia, of the severity of that encountered in thyroid-fed rabbits, was observed.

It is not possible at this stage, however, to say how far the other effects of thyroid feeding may contribute to the production of the intense hypoglycæmia observed in thyroid fed animals. The recent work of Cramer(18), and of Kojima(19) suggests that excessive thyroid feeding leads to hypertrophy of the islet tissue of the pancreas, accompanied by atrophic changes in the pituitary body, while Buru(16) has demonstrated the antagonism between pituitary extract and insulin. It may well be, therefore, that the operation of these factors, in conjunction with the depletion of glycogen reserves, will account for the dramatic and paradoxical results here described.

It seems clear that maintenance of the normal blood sugar level involves the balanced action of a number of opposing factors. Just as the liver compensates for a fall by accelerated output of glucose, the pancreas compensates for a rise by accelerated output of insulin. Even under normal conditions, a temporary over compensation may occur in one direction or the other, but the balance is maintained, on the whole, with remarkable accuracy. The balance can be disturbed by the influence of other endocrine glands, and of various drugs. Adrenalin, and sympathetic stimulation, certainly accelerate the output of glucose from the liver, and there is some evidence (Clark(15)) that vagal stimulants cause the pancreas to secrete insulin.

The thyroid hormone sensitises the liver to influences promoting glycogenolysis, among which we must presumably include the secretion of insulin *per se*, for glycogenolysis may apparently be elicited without the previous occurrence of hypoglycæmia (Fig 1 B). Whether the insulin acts here as a direct liver stimulant, or by exciting the suprarenal glands to output of adrenalin, is a point on which no complete evidence is available, but Houssay's(17) demonstration, that the secretion of adrenalin is accelerated by injecting insulin, is suggestive.

## SUMMARY.

1. A secondary hyperglycæmia is observed to follow the injection of glucose or of a small dose of insulin, into the thyroid-fed rabbit whose liver still contains glycogen.
2. When the liver is depleted of glycogen, a small dose of insulin, or an injection of glucose, produces a fatal hypoglycæmia.
3. The bearing of these observations on the mechanism of blood-sugar regulation is discussed.

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## THE COURSE OF THE BLOOD OF THE RENAL ARTERY. BY J. N. LANGLEY.

(From the Physiological Laboratory, Cambridge.)

THERE are many accounts of the blood flow through the kidney. A few only need here be mentioned. Bowman in 1842 described the renal artery as conveying a small quantity of blood direct to the capsule, to the surrounding fat and to the coats of the larger vessels. Except for this small quantity, he considered that the blood conveyed by the renal artery passed through the glomeruli. Virchow in 1857 described the arteries of the boundary layer of the human kidney as giving off small arteries to the medulla, the arteries soon breaking up into a bundle of vessels. In one case of amyloid degeneration of the glomeruli, he stated that the injection mass did not pass through the glomeruli, but that, nevertheless, the arteriæ rectæ (subsequently called art. rect. veræ) were completely injected. He gave figures "drawn as accurately as possible from injected specimens" showing in the clearest way small arteries passing directly to the medulla. This view of a collateral circulation in the kidney was supported by Ludwig, Heidenhain and others.

Virchow had pointed out that it was difficult to follow the arterial branches in a fully injected kidney, and in view of this difficulty Golubev in 1893 used silver nitrate as an injection material, thus allowing arteries and veins to be more easily distinguished. He confirmed the existence of art. rectæ veræ, in not inconsiderable numbers, and stated that in some cases the afferent artery of a glomerulus gave off, close to the glomerulus, a branch supplying blood direct to the kidney tubules. A diagram of this by-pass to the capillaries of the cortex is given in H. Meyer and Gottlieb's *Experimentelle Toxicologie*, 6th edit. p. 100, 1922, and well illustrates the control which might be exercised on the glomerular blood flow. It may be also mentioned that Kolliker and others described small branches of the cortical arteries as running direct to the capsule, and that some observers have found anastomoses between arteries and veins in the capsule of the kidney (Geberg) and in the boundary layer.

The question of direct medullary arteries was taken up by Huber (1907). He macerated thick sections of injected kidneys (rat, guinea-pig, rabbit, dog) and found that in nearly all cases each small artery passing

to the medulla could be seen to arise from the efferent vessel of the glomerulus (arteriola recta spuria). In the dog he now and then found true direct arteries, but these formed a very small per cent. of the medullary arteries. Occasionally, particularly in the dog, a branch of a cortical artery appeared to anastomose with capsular arteries. He concluded, as had Bowman, that "practically" all the blood passed through the glomeruli. Gerard (1911) examined serial sections of portions of the kidney (rat, rabbit, man) and came to the conclusion that no direct medullary arteries existed. Recently Traut (1923) has made observations on the kidney of man. He macerated the kidney after injecting it from the ureter with a special mixture, and occasionally found a direct medullary artery, but this was rare for in a whole kidney he found six only.

In many investigations on secretion by the kidney tubules, it is essential to know whether any appreciable portion of the blood of the renal artery passes to the tubules without passing through the glomeruli. Past observations show that agreement in this point is not likely to be reached by the method of injecting all the vessels. Agreement as to the rarity of arteriolæ rectæ veræ might no doubt be reached by repetition of the method of maceration, but, so far, this rarity has received very scanty recognition by histologists and the maceration method has not been applied to other disputed questions of the kidney circulation.

The following method is simple and gives, I think, decisive results on most, if not on all, the questions.

The kidney immediately after death is washed out from the renal artery with warm Ringer's fluid to which a drop or two of amyl nitrite is added. A 10 p.c. suspension of rice starch grains in similar warm Ringer's fluid is then injected. The artery is tied, and the kidney placed in formaline (10 p.c.) for a day, and for a day in 50 p.c. alcohol. A piece is cut out of the centre parallel to the medullary rays, soaked in gum and thick sections cut frozen. The rest is cut into slides about 1 mm. thick. The sections and slices are placed in an aqueous solution of iodine dissolved in potassium iodide which quickly stains the arteries black the rest of the tissue brown. If it is required to examine without delay, the sections and slices are placed for 5 to 10 minutes in acetone or absolute alcohol, and as soon as the brown stain has become light-yellow the sections (if necessary further dehydrated) are cleared in clove oil, passed through xylol, mounted in balsam, and examined under the microscope; the slices are transferred to water and examined with a lens. If it is not required to examine at once, it is better to put the sections



and slices in water, until the brown or yellow stain has disappeared, thus, according to the duration of stay in iodine, may be any time from an hour to a day. The arteries are then a deep black on a colourless background. When mounted they decolorise in two to six days but they can be re-stained at any time.

Sections dried on the slide do not decolorise, they show the position of the arteries, but are useless for microscopic examination. Slides dehydrated and mounted in balsam without a cover slip may last for a month or two.

Slices stained with an alcoholic solution of iodine, partially decolorised in alcohol and cleared in clove oil make, when freshly prepared, effective preparations for low power magnification (cf Fig 2).

I have examined sections and slices of a number of kidneys of the rabbit and one or two of the cat, dog and guinea-pig. The fat tissue in the hilum and around the kidney was generally removed before cutting sections and examined separately. We may consider first the blood of the renal vessels which do not pass to the capillaries of the kidney tubules. In arterial injections, all the arteries of the tissues of the hilum and of the upper half of the ureter are injected. The injection of the capsule and of the fat around the kidney varied and was never complete, the variation may have been due to differences in injection pressure, but the fat no doubt receives blood from other sources than the renal artery. The renal artery supply to the capsule and fat is peculiar. At wide intervals an interlobular artery runs through the cortex with little or no decrease in size and arriving at the surface sends a branch or two at once to the capsule (cf Fig 7), and then runs outward, dividing and supplying a considerable mass of fat. So far as I have seen there are only six to eight of these in the whole kidney. In their proximal course they commonly give off arteries to glomeruli. The best way to find them is to turn back the fat gently from the middle of the convex surface of the kidney, flooding with iodine as the fat is separated. The arteries are then seen issuing from the kidney, and thin slices containing them may be cut and treated for microscopic examination.

In the two injections made from the renal vein, the veins of the hilum tissue and of the ureter were well injected and one or two small capsular veins. The arteries and veins mentioned above are all small and blood which passes through them can only be a very small proportion of that of the renal vein.

On naked eye examination of the mounted sections and slices from which the staining of the tubules has been removed by water, it is seen that whilst the arteries of the boundary layer, the interlobular arteries

and the arteries to the glomeruli are dense black, there is hardly a trace of colour in the medulla. The usual appearance is that of Fig. 1. In this at *a* there is one small injected vessel running into the medulla, and one only. In a good many sections there are none, but there may be two; the most I have seen is three.



Fig. 1.

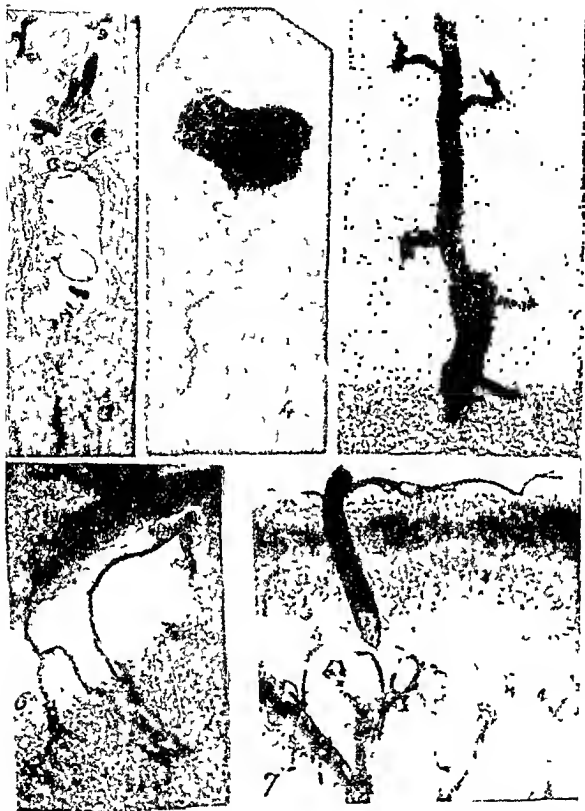
Fig. 2.

Fig. 1. Cortical (interlobular) arteries. At *a* a small direct artery, at *b* a small artery of the hilum system.

Fig. 2. Boundary layer artery giving off interlobular arteries and these giving off afferent glomerular arteries.

As a rule the whole course of a direct medullary artery cannot be seen unless the section is thick. The usual appearance is shown in Fig. 3 (which is *a* of Fig. 1 more magnified) and in Fig. 4. Thus usually, to make sure of their origin and course several sections must be examined. The whole course can however be seen in thick sections. They usually arise direct from a large artery. I have only seen one instance of a direct medullary artery arising from the afferent artery of a glomerulus. This is shown in Fig. 6.

Besides these injected arteries there are sometimes single small injected vessels in various positions in the medulla extending for  $\frac{1}{4}$  to  $\frac{1}{2}$  its length. When they occur there are a few grains of starch to be



Figs. 3-7 Figs. 3 and 4 Arterio rectae vere as ordinarily seen in sections. Fig. 3 is a of Fig. 1, the artery arises from a small artery in the connective tissue surrounding a larger artery, it divides into three branches, not distinct in the reproduction. Fig. 6 Thick section. Arterio rectae vere arising from an artery giving off two branches each ending in a glomerulus. Fig. 5 Part of injected interlobular artery, each branch ending in a glomerulus, to show that if there were side branches they would be visible. Fig. 7 Interlobular artery (perforating artery) running to the surface of the cortex, sending a branch on each side to the capsule, it continued on into the fat and there supplied a considerable mass of fat tissue.

seen here and there in most of the capillaries. They are caused I think by the smaller starch grains passing into the capillaries under high pressure of injection and accumulating in one or other blocked capillary. But if every medullary injected vessel were a small artery, the amount of blood passing through them would be infinitesimal compared with that passing through the glomeruli.

The peripheral part of many of the interlobular arteries and of the glomeruli arising from them are included in a rather thick section and it is obvious that the afferent glomerular artery gives off no branches conveying blood directly to the capillaries of the tubules. Since the afferent arteries are at different levels it is difficult to obtain a photograph showing how certainly the small arteries can be traced. But Fig. 5 will perhaps be sufficient to indicate that if there were a direct branch it could be easily seen. In the boundary layer the afferent glomerular arteries are much more irregular than they are peripherally, some of them are short, some, especially in the dog, are long, but in a sufficiently thick section it can be seen that, with the rare exceptions mentioned above, every branch of an artery ends in a glomerulus.

The most convincing specimens, both as regards direct arteries to the medulla and direct arteries to the cortex are the slices of kidney, stained with alcoholic solution of iodine, partly decolorised, cleared in clove oil and examined under a low power of a stereoscopic microscope. These show the manner in which a number of interlobular arteries arise from an artery at different levels in the boundary layer, the numerous afferent glomerular arteries which in the lower part of the cortex run towards the medulla and the irregular course and length of these. They show also that in the deeper cortical region it is not infrequent for an afferent glomerular artery to divide, each branch ending in a glomerulus and that this sometimes occurs in the more superficial region. Occasionally the branching of the artery occurs close to the glomerulus; in a section, one of these branches would probably be cut, and it is possible that the direct artery to the capillaries described by Golubev was of this kind. Fig. 2 is taken from a slice treated as just described. In the specimen every arterial branch could be followed, under the stereoscopic microscope, to a glomerulus. Fig. 8 is a sketch of part of an interlobular artery kindly drawn for me by Dr Jarisch from a similar specimen.

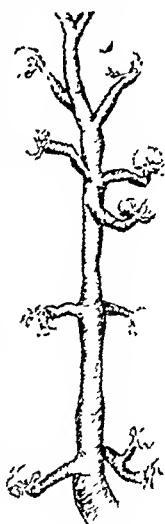


Fig. 8.

The results I think show that in the rabbit, cat and dog there are no direct arteries to the cortical capillaries and that so far as there are direct anastomoses between arteries and veins the connecting vessels are of capillary size. I agree with Huber and Traut that the number of small arteries running direct to the medulla is trivial though these arteries are not so rare as they describe. In the guinea-pig, the glomeruli are very close together and I have not examined them in much detail, but, so far as I have seen, the arterial connexions are the same as in the rabbit.

On the basis of embryological investigation Traut regards the rare direct medullary arteries as arising from atrophied glomeruli. There are some features of these arteries which lead me to think that most of them do not belong to the circulation of the kidney tubules but to the blood supply of the renal vessels and of the tissues accompanying them. In Fig. 2 it will be seen that the small direct medullary artery is not in the position described for the *arteriolæ rectæ veræ*, but arises from a small artery in the connective tissue sheath of a relatively large artery. In Fig. 1 there is a small artery at *b* in the connective tissue of the hilum. An artery in this position not infrequently (in proportion to the total number of direct arteries) sends a branch which curves into the medulla at the base of the papilla, and divides in two or more small branches running towards the apex of the papilla.

The renal vein (rabbit) was only injected twice. The interlobular veins and numerous medullary veins were well injected; from the interlobular veins the starch penetrated a short way into the capillaries joining it, but no other part of the kidney substance was injected.

#### SUMMARY.

The distribution of the branches of the renal artery can be readily determined by injecting it with a suspension of rice starch grains and staining sections and slices with iodine.

The renal artery supplies blood, as originally described by Bowman, to the tissues of the hilum, the capsule and the surrounding fat. The supply to the capsule and the surrounding fat appears however to be partial and variable. The supply is by six or eight interlobular arteries which run through the cortex with little or no decrease in size. The renal artery also supplies blood to the ureter. The renal vein receives blood from the same areas.

The cross-section of all the arteries which do not run to the kidney tubules is very small compared with that of the interlobular arteries

but it is conceivable that in certain conditions the quantity of blood passing by them to the renal vein is not negligible.

No direct branches to the capillaries of the kidney tubes are given off by the afferent glomerular arteries.

The direct arterial branches (*arteriolæ rectæ veræ*) to the medulla are less infrequent than are described by Huber and by Traut, but they are so rare and so small that they are certainly negligible in physiological investigations and they cannot be regarded as affording a collateral circulation. It is suggested that in origin most of them are not part of the system of vessels supplying the kidney tubules, but are aberrant parts of the vessels supplying the large arteries and veins.

The micro-photographs were taken by my laboratory assistant Mr Freeman. They are of the kidney of the rabbit; and of specimens injected from the renal artery.

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# THE CIRCULATION IN SKELETAL MUSCLE, CHIEFLY IN RELATION TO THE EFFECT OF THE DEPRESSOR NERVE OF THE RABBIT.

By A. JARISCH (*Fellow of the Rockefeller Foundation*)

(*From the Physiological Laboratory, Cambridge*)

THE view that stimulation of the central end of the depressor nerve of the rabbit causes vaso dilatation in skeletal muscle rests mainly on indirect experiments. The most direct are those of Bayliss<sup>(1)</sup>. He cut off the feet of an anæsthetised rabbit, removed the skin of the leg as far as the upper part of the thigh and placed the skin free part of the leg in a plethysmograph. He found that stimulation of the depressor caused an increase of the volume of the limb, but this was sometimes preceded by a decrease. The decrease he considered to be passive and due to the muscle vessels having a longer latent period than those of the viscera. The experiment is fairly conclusive, but as the limb contained bone and fascia and the increase of volume seems to have been small compared with the volume of the limb (the plethysmograph was not calibrated) and since Bayliss by another method failed to obtain evidence of vaso-dilatation in the muscles, there was room for further experiments. At Prof. Langley's suggestion I have investigated the volume changes in a single muscle—the gastrocnemius—caused by depressor stimulation in various conditions and have also made observations on some other ways of affecting the circulation in the muscle.

## *The effect of the depressor nerve in various conditions*

**Method.** The experiments were made on rabbits anæsthetised with urethane and C.E., both vagi were cut. The external saphenous vein was tied, the soleus muscle cut from the gastrocnemius and slit up, the Achilles tendon cut and a knot tied on the end with rather thick string so as to form a projecting ring. The gastrocnemius muscle was placed in a thin rubber tube closed at one end (a condom). The tube was tied round the tendon below the knot, a loose end being left. The muscle was then placed in a metal plethysmograph of elliptical cross section, the loose end of the string drawn through the neck of the plethysmograph.

and fixed by a rubber cork carrying the glass-tube connection with the recorder. The end of the rubber tube was turned back over the edge of the plethysmograph and tied. The soleus muscle and the skin then were sewed round the base of the plethysmograph as to form a cuff. A diagram of the arrangement is shown in Fig. 1. Only 10-15 c.c. of

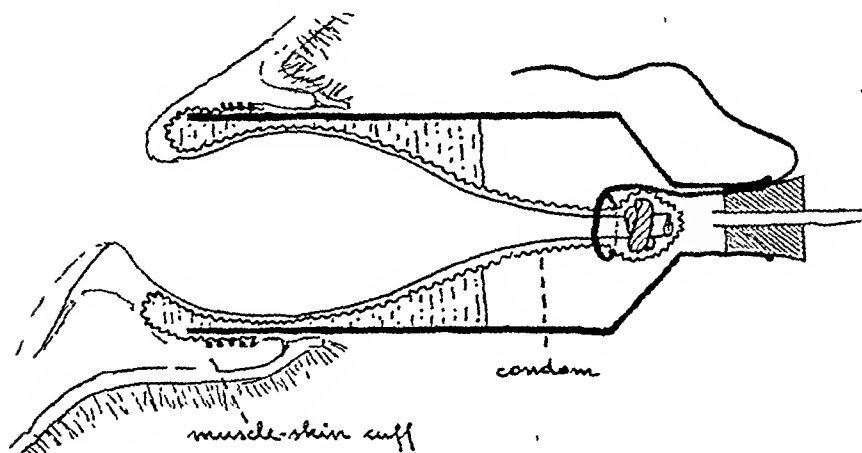


Fig. 1. Diagram of gastrocnemius muscle in the plethysmograph.

Ringer were poured into the plethysmograph (to avoid any pressure on the muscle veins) but sufficient to fit the rubber close round the base of the muscle. The record was taken by a specially constructed and calibrated tambour; an increase of 0.1 c.c. raised the lever 8.5 mm., and this caused an increase of pressure inside the plethysmograph of only 1.6 mm. water. The volume of the muscle inside the plethysmograph was generally about 12 c.c.

Stimulation of the depressor in my experiments always caused a primary increase of volume of the gastrocnemius coincident with the fall of arterial blood-pressure. The degree of increase varied considerably in different rabbits and, so far as I could determine, the variation was due to intrinsic and not to experimental conditions. The rate of increase of volume varied independently of the rate of fall of blood-pressure.

The volume changes obtained are of two types:

1. The most common form is that the volume, though increasing with the fall of blood-pressure, begins to decrease when the pressure is about at the lowest level and gradually returns to about the original volume although the stimulation continues and the pressure remains low (Fig. 2 a).



2. Less commonly, the volume increases at first, declines slightly only throughout the period of stimulation and decreases relatively quickly as soon as the stimulation ceases (Fig. 2 b).

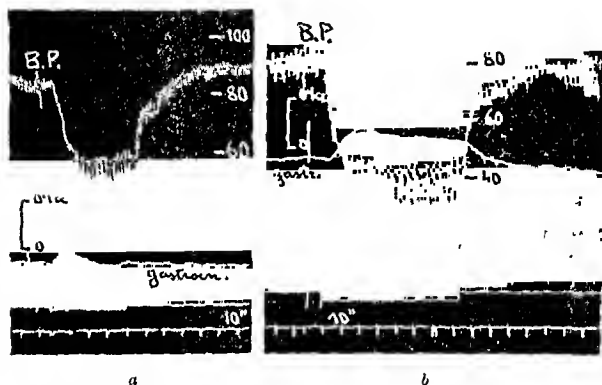


Fig 2. Effect of the depressor on muscle volume (a) type 1, (b) type 2.

Intermediate forms between these two types sometimes occur, but not in any one experiment.

In order to determine how far the changes of volume of the muscle are due to its nervous connection and how far they are passive and caused by variations in arterial or venous pressure, the depressor was stimulated in various conditions.

*Obstruction of the venous outflow.* The vena cava was clamped above the iliac veins and the depressor stimulated. Clamping the vena cava caused, as was to be expected, an increase of volume in the gastrocnemius. The increase having reached a certain degree, usually remained at this during the period of obstruction, as soon as it had reached a fairly constant level (18 to 20 sec.) the depressor was stimulated. It caused constantly a further increase of volume of the muscle (see Fig. 3). The volume began to return to its previous level during the stimulation although the blood-pressure was still low, i.e. the curve was generally of type 1 mentioned above. Occasionally the vena cava was clamped for 3-5 minutes and the depressor stimulated several times; each stimulation caused an increase of muscle volume. Thus the increase of volume is due, in part at least, to dilatation on the arterial side.

*Section of vaso-constrictor nerves of the muscle.* The lumbar sympathetic was excised and the depressor then stimulated. The only effect then obtained was a decrease in the volume of the muscle. In one experiment the depressor was stimulated before and after section. Fig. 4a shows the effect of stimulation before and Fig. 4b the effect after the section. It will be seen that the decrease in volume, which occurred is practically the same in both cases, indicating that the decrease of volume in the type 1 curve is a passive effect of the fall of blood-pressure.

*Evisceration and the use of a compensator.* If the fall of blood-pressure is the cause of the decrease of muscle volume occurring in the latter part of the type 1 curve it should be absent if fall of pressure is prevented. A compensator filled with Ringer's fluid was used in the manner given by Roberts. When this was connected with the carotid or the femoral artery a large quantity of Ringer's fluid passed into the circulation but it did not prevent a large fall of blood-pressure on stimulation of the depressor. A great reduction of the fall was however obtained by evisceration and connecting the compensator with the superior mesenteric artery. The depressor then caused a simple increase of muscle volume continuing for 20 seconds or more after the stimulation had ceased. It may be mentioned that after evisceration but previous to the use of the compensator the normal type 1 curve was obtained.

*Section of the spinal cord in the lumbar region.* The absence of increase of muscle volume when the depressor was stimulated after section of the abdominal sympathetic shows that the increase ordinarily occurring is not due to reflex antidromic vaso-dilatation. To test this in another (though less conclusive) way, the depressor was stimulated before and after section of the spinal cord between the 4th and 5th lumbar segment.

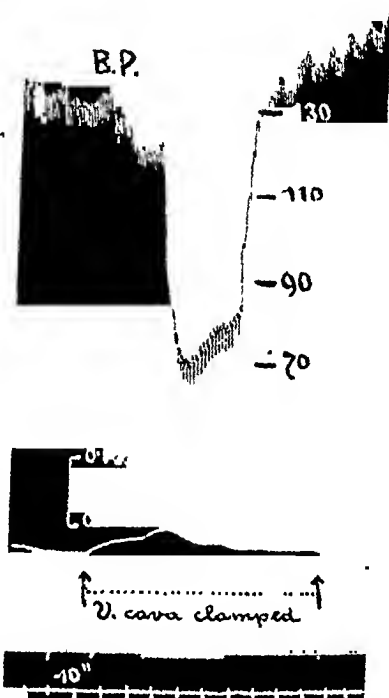


Fig. 3. Between the arrows the vena cava was clamped; nevertheless the depressor produces an effect on muscle volume of the type 1.

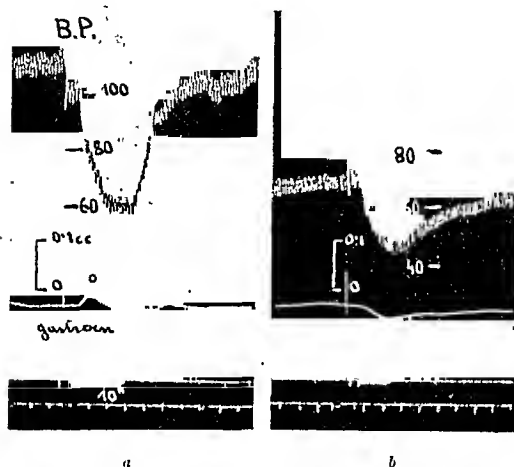


Fig. 4. Depressor on muscle volume (a) before, (b) after excision of the sympathetic in the lumbar region.

Section of the spinal cord left the form of the curve of muscle volume unaltered (Figs. 5 a and b).

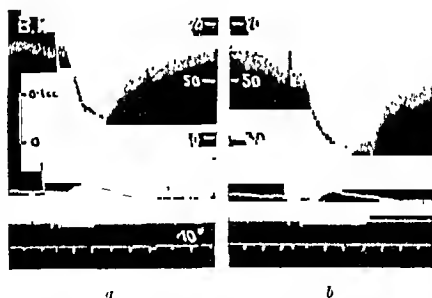


Fig. 5. Depressor on muscle volume (a) before, (b) after section of the spinal cord between the 4th and 5th lumbar.

*Mechanical lowering of arterial pressure.* If reflex dilatation caused by the depressor were an active effect, i.e. accompanied by an increase of diameter of any of the vessels apart from internal pressure, it might cause some passage of blood into the muscle when the arteries outside it were nearly empty. To test this the aorta was clamped below the kidneys and the depressor then stimulated; no change in muscle volume was however found.

The foregoing experiments show, I think, conclusively that the depressor causes dilatation of muscle vessels. They confirm Bayliss' deduction. There is, however, one difference in result. Bayliss, as I have mentioned above, found sometimes a primary decrease of volume. In my experiments the primary effect was always an increase, coincident with the fall of blood-pressure; there is then no need to consider that the muscle vessels have a longer latent period than the visceral vessels. Bayliss(3) considered that the fall of pressure caused by the depressor was partly due to antidromic vaso-dilatation. This I do not find to be the case in the rabbit.

The promptness of the increase of muscle volume in normal conditions and of the decrease after section of the sympathetic shows also that neither of these changes is due to any alteration of hormone content of the blood.

The decrease of the volume of the muscle in type 1 curve after the blood-pressure has reached its lowest limit, and while the pressure is still low, shows that in the redistribution of blood which is caused by depressor stimulation, the blood lost from the arteries has a *short stay* only in the muscle, probably it accumulates in the visceral vessels or in the veins. It is curious however, that the volume of the muscle does not sink below its original volume, and that the decrease of volume caused by fall of arterial pressure should be exactly compensated by depressor dilatation.

#### *Other observations.*

(1) Stimulation of the central end of the *vagus* has a much more variable effect on the muscle volume than stimulation of the depressor. Change in respiration and often movements of the animal disturb the vascular results; it needs very deep narcosis to prevent them and the narcosis on its part alters the vaso-motor conditions. If stimulation of the *vagus* causes a fall of blood-pressure, the muscle volume generally increases, producing a curve similar to the type 1 of the depressor; if on the other hand the blood-pressure rises, the muscle volume tends to decrease. The *vagus* effect depends in some undetermined way on the

depth of narcosis and the strength of the stimulation. Langley(2) generally found that a weak current tended to cause a rise of blood-pressure and a strong current a fall of pressure. In two of my experiments weak currents caused a fall and stronger currents a rise.

(2) Experiments in which the *aorta* was temporarily closed in the abdomen showed regularly a marked rise of volume as soon as the blood stream was allowed to pass (see Fig. 6). This phenomenon first described by Bayliss was explained by him as a local reaction to diminished stretching of the arterial wall, while Anrep(4) related the dilatation to a production of asphyctic metabolites. Against the latter view is the fact that closing the vena cava even for as long a period as five minutes has comparatively little effect on the muscle volume although the suspension of the venous flow must have caused considerable local asphyxia, and further that the venous obstruction has very little after-effect.

(3) Experiments with *adrenaline* showed that the changes in volume of the gastrocnemius muscle agrees exactly with the change of the whole limb as described by Oliver and Schäfer(5). Sometimes and chiefly after small doses of adrenaline the muscle dilates passively, its curve running parallel to the blood-pressure. After larger doses, a primary increase is followed by a sudden decrease obviously due to a constriction of the muscle vessels. In experiments on the cat, 1 c.c. adrenaline 1 to 200,000 caused a marked increase of the muscle volume while the blood-pressure was falling, confirming the statements of Hoskies, Gunning and Berry(6) on the skinned leg of the cat.

(4) *Asphyxia* correspondingly lowers the muscle volume as the blood-pressure rises. In preparations in good condition when there is a considerable rise of pressure the decrease of volume turns immediately to a remarkable increase as soon as breathing is allowed (Fig. 7 a). Obviously the blood-pressure dilates the muscle vessels as soon as the central asphyctic stimulus ceases and this effect is reinforced by the increased output of the heart which recovers from its asphyctic failure as soon as the oxygenised blood reaches it. This is the explanation of the after rise of blood-pressure sometimes following asphyxia given by Starling and Kaya(7). In cardiometer experiments, which I have made

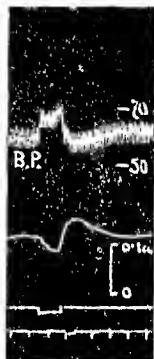


Fig. 6. Effect of clamping the aorta for a period of 12'; sciatic cut.

with Dr Helene Wastl, the heart volume dropped to its primary level coincident with the after rise. Further the conditions in the chest at the end of the asphyctic period when normal respiration begins are favour-

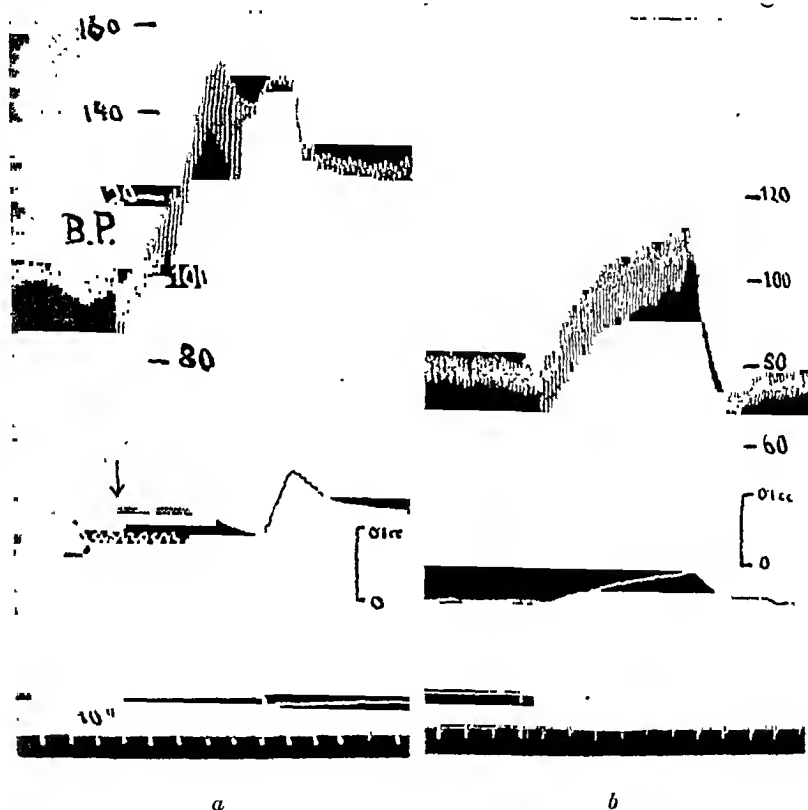


Fig. 7. Effect of asphyxia (a) normal, (b) after cutting the sympathetic.

able to an increase of the output of the heart. After cutting the sympathetic, the muscle responds in asphyxia by a simple increase of volume (Fig. 7 b) which is obviously passive and shows that in the normal muscle the decrease of volume is of nervous origin and not, or not altogether caused by a greater activity of the adrenals.

(5) Finally I give in Fig. 8 an illustration of the fact stated by Gaskell(8) that severing the nervous connections of a muscle causes vaso-dilatation of short duration, since so far as I know no graphic record of this fact has been published. Cutting the sciatic nerve gives the same result.

## SUMMARY.

Plethysmograph experiments were made on the gastrocnemius muscle of the rabbit in order to determine how far the effects described by previous observers in the skinned limb hold for muscle; the following results were obtained:

1. Stimulation of the depressor causes a primary increase of volume of the gastrocnemius muscle of the rabbit. This increase is generally followed by a decrease although the stimulation continues; the decrease is passive and due to the fall of blood-pressure.

2. Obstruction of the venous outflow does not prevent the effect of the depressor on the muscle volume indicating that the change of volume occurs chiefly on the arterial side.

3. After section of the sympathetic no dilatation occurs but only passive shrinking coincident with the fall of blood-pressure.

On the other hand cutting the spinal cord at the 4th lumbar segment does not affect the influence of the depressor on muscle volume. Thus the dilatation is not due to reflex antidromic action but to inhibition of the sympathetic tone. The dilatation is not an active process and is absent when the arteries are empty.

4. Stimulation of the vagus causes in general an increase or decrease of muscle volume according as the blood-pressure rises or falls.

5. Asphyxia causes a decrease of muscle volume which turns generally to an increase as soon as normal breathing is allowed; after cutting the sympathetic only passive increase occurs due to the asphyctic rise of blood-pressure.

I wish to express my thanks to Prof. Langley for suggestions and kind advice during the course of this work.

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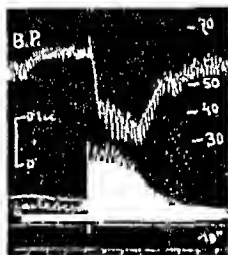


Fig. 8. Cutting the spinal cord between the 4th and 5th lumbar in deep anaesthesia (from the same experiment as Fig. 5).

# THE INFLUENCE OF TEMPERATURE ON THE EQUILIBRIUM BETWEEN OXYGEN AND HÆMOGLOBIN OF VARIOUS FORMS OF LIFE.

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It has been shown by J. Barcroft and H. Barcroft(1), that the affinity of Arenicolan hæmoglobin for oxygen differs from that of man, and Anson, Barcroft, Mirsky and Oinuma(2) pointed out the very great difference in relative affinities of Hb for O<sub>2</sub> and CO in different animals. Taking these statements as basis, we attempted to compare oxygen dissociation curves of solutions of hæmoglobin of different forms of life under identical conditions. Hartridge and Roughton(3) state, that dilute solutions of hæmoglobin in a concentration 1 : 1000 give rectangular hyperbolæ as dissociation curves, and that the equilibrium between oxygen and hæmoglobin in dilute solutions depends only on the hydrogen ion concentration and the temperature, and is not influenced even by large amounts of salts present in the solution.

*Methods.* Solutions of hæmoglobin were prepared simply by dilution of blood with distilled water. Human blood was withdrawn from a vein and defibrinated; in some cases drops of blood, obtained by pricking a finger, were allowed to fall into distilled water. The concentration of Hb of such solutions (1 : 280 to 1 : 1500, to suit the thickness of the absorption cells used) was ascertained by comparing them with solutions of known concentration. Frog's blood was obtained by amputating the fore-leg and bleeding the animal; for obtaining solutions of Hb from *Planorbis* an incision was made in a snail's foot and the blood dropped into distilled water. The solutions were filtered and centrifuged, when not quite clear. In order to prevent bacterial and enzymatic action, a small amount of KCN (1 drop of a 2 p.c. solution to 20 c.c. of the hæmoglobin solution) or NaF (0.1 p.c.) was added. Solutions were buffered with Sørensen's M/15 phosphate mixture  $pH = 7.4$ ; one volume of buffer to 100 volumes of the hæmoglobin solution was employed. The alteration of  $pH$  of phosphate mixtures with temperature is negligible, as shown by Walbum(4).

<sup>1</sup> Travelling Fellows of the Rockefeller Foundation.



Determinations of percentage saturation with oxygen were made by using Hartridge's reversion spectroscope. The method has been described by Hartridge and Roughton<sup>(3)</sup> and J. Barcroft and H. Barcroft<sup>(4)</sup>. Our arrangement was in the first experiments the same as described by the authors last quoted. The solution of hæmoglobin was put into a Barcroft saturator, the rubber stopper of which was pierced by a short glass test tube of about the same internal thickness as the half of the diameter of the neck of the saturator. The tube contained the same solution of hæmoglobin, saturated with carbon-monoxide. When the saturator was reversed with the neck downwards and put before the slit of the spectroscope, the beam of light passed both solution of oxyhæmoglobin and carboxyhæmoglobin. This arrangement yielded us less consistent results owing to the difficulty of adjusting the neck of the saturator exactly in the same place before the slit of the spectroscope.

We then reversed the test tube, the sealed end outwards, which now contained oxyhæmoglobin and placed it in a wooden block with a vertical and a horizontal hole, which was fastened on the supporting board of the spectroscope. The solution of COHb was put into an absorption cell before the slit. This arrangement proved to be satisfactory for experiments, in which the equilibrium between hæmoglobin and oxygen was obtained at room temperature. We used it also in determining equilibria at higher temperatures, but it involved a grave error, since the temperature of the fluid inside the saturator, taken out from the water bath falls very rapidly. However, it was possible to calculate corrections for the increase of the saturation of hæmoglobin with decrease of temperature, when the oxygen dissolved in the solution became attached to hæmoglobin. These corrections were very great and affected the accuracy of the results. We preferred therefore to carry out determinations at the same temperature, at which the equilibrium has been obtained.

In our final and decisive experiments we used cells of a small internal thickness. These were made by cementing together strips of glass plate with Khotinsky cement. The measurement of the inner space of the cell for HbO<sub>2</sub> were 30 mm. × 10 mm. × 1.5 mm. The cell (c) was connected with the saturator (s) by means of a wide rubber pressure tubing (r) as shown in Fig. 1. In order to obtain a water- and air-tight connection, the rubber was bound over with brass-wire and protected with a

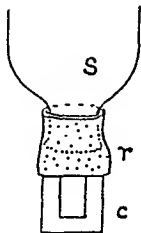


Fig. 1.

thin layer of paraffin or collodion. The solution of COHb in the other cell was covered with a layer of paraffin oil and closed with melted paraffin wax. A glass jar containing 1 litre of water was put before the spectroscope. To the bottom of the jar a metal frame was fixed, allowing both cells to be put always in exactly the same position. The cell containing COHb remained in its place during the calibration and actual determination. The saturator was fixed during the determination with a clamp. The glass jar was filled with water of the required temperature, which was kept constant by stirring the water bath and adding small amounts of hot water.

Calibration curves for determining the percentage saturation with  $O_2$  are made as follows. Put the solution of hæmoglobin, diluted to a concentration suitable for spectroscopical examination, into the vessel, test tube or cell, in which the actual determinations are to be made, and the same solution saturated with CO into the other vessel. Place both in the path of light before the spectroscope slit and take readings. These correspond to 100 p.c. saturation of oxyhæmoglobin. Make dilutions of the same solution of hæmoglobin with distilled water, *e.g.*  $1/5$ ,  $1/3$ ,  $1/2$ ,  $2/3$ ,  $4/5$  corresponding to respectively 20, 33.3, 50, 66.6 and 80 p.c. saturation of  $HbO_2$ , put them into the cell and take readings again. The differences in scale units between 100 p.c. and the readings for each dilution are plotted against the corresponding percentages of  $O_2Hb$  and thus the calibration curve is obtained. It is of course different for hæmoglobins from species with different spans. For actual determination a correction for the presence of the broad absorption band of reduced hæmoglobin is necessary. We used a correction table worked out by Mr Roughton. The corrected curve is obtained by shifting points of the calibration curve on the abscissa (per cent. saturations) towards the zero point (100 p.c. saturation) for the following distances, expressed in per cent. units of the abscissa: 8.5 at 20 p.c. = 28.5, 9 at 30 p.c. = 39, 9 at 40 p.c. = 49, 10 at 50 and 60 p.c. = 60 and 70 respectively, 9 at 70 p.c. = 79.

Determinations of percentage saturation with Hartridge's spectroscope are of quantitative value only between 70 and 30 p.c. We aimed therefore at obtaining points of calibration curves between these limits as accurately as possible and determined them several times for the different hæmoglobins. Scale readings on the spectroscope gauge were different for both observers, but the differences, and thus the calibration curves obtained, were practically identical. We took for each point usually the average of ten readings.

Calibration curves were made only at room temperature. The  $\alpha$  bands of both oxy- and carboxyhæmoglobin shift towards the red with rise of temperature per degree about  $\cdot 28 \text{ \AA}$ , as shown by Hartridge(5). Therefore in measurements at higher temperatures we corrected our zero readings (100 p.c.  $\text{O}_2\text{Hb}$ ) by adding for every  $10^\circ$  increase of temperature  $2\cdot 8 \text{ \AA}$ , corresponding to 2.5 scale units on our instrument. The average error of our mean scale readings is less than 1 p.c., that is to say, it is between 0.5 and 1.0  $\text{\AA}$ , and the error of oxygen percentage determinations between 30 and 70 p.c. is about  $\pm 3$  p.c., if we make an allowance for slight differences between determinations of two observers.

In order to obtain different oxygen tensions, the saturator was evacuated by means of a water pump to a calculated pressure of air and then filled with  $\text{N}_2$ . The tensions of  $\text{O}_2$  were determined by the Haldane apparatus for gas analysis. When working with human hæmoglobin, very low tensions of oxygen were required, which could not be determined by gas analysis. In this case we evacuated the saturator with a Geryk pump, filled it then with pure hydrogen<sup>1</sup> and forced into it a measured amount of air from a gas burette. The tensions of  $\text{O}_2$  were then calculated. The saturator was exposed in a water bath to different temperatures and rotated. The time of exposure varied from five minutes, when using higher  $\text{O}_2$  tensions, to ten minutes, when using low tensions. Several measurements of the temperature in the saturator proved this time to be sufficient for obtaining the required temperature. We aimed at shortening all our procedures, in order to prevent a too fast breakdown of hæmoglobin. In order to get information about the changes in the solution, we took zero point readings during the course of the experiment and after finishing it. Sometimes, especially when working with frog's blood, we obtained finally a much lower value for the zero point than at the start, indicating a breakdown of the pigment. Such series of determinations were usually discarded, or, when the change did not very much exceed the limits of experimental error, a correction was made for the change of the zero point, taking the mean of the initial and final reading.

*Calculation of equilibrium constants and temperature coefficients.* We tried to determine oxygen dissociation curves at  $10^\circ$ ,  $20^\circ$ ,  $30^\circ$  and  $40^\circ \text{ C}$ . or, in our final experiments, at  $15^\circ$ ,  $25^\circ$  and  $35^\circ \text{ C}$ . The principles, on which our curves are drawn, are those deduced by A. V. Hill(6) from the laws of mass action, with the difference, that we plotted on the ordinate percentage saturations of oxyhæmoglobin, as a matter of con-

<sup>1</sup> We are indebted to Messrs M. L. Anson and A. E. Mirsky who kindly allowed us to use the hydrogen which they brought to the high state of purity.

venience, and accordingly calculated the values of  $K$  in another way, as follows: We call the percentage content of reduced hæmoglobin  $y$ , the percentage of oxyhæmoglobin  $(100 - y)$ , the tensions of oxygen in mm. of Hg  $x$ ,  $a$  the distance of the asymptote from the zero point of the hyperbola and therefore  $(x + a)$  the distance of any point of our abscissa from the vertical axis. The formula of a rectangular hyperbola is  $xy = K$  and  $a$  being  $K/100$ , hence

$$y(x + a) = K = 100a,$$

$$a = \frac{yx}{100 - y},$$

and

$$K = \frac{100yx}{100 - y}.$$

The value of  $x$  at 50 p.c. saturation equals  $K/100 = a$ ,

$$K = \frac{100x \times 50}{50},$$

$$x = \frac{K}{100} = a.$$

This value has been called by Krogh and Leitch (7) the "tension of unloading" of the hæmoglobin,  $t_u$ , and gives a good index when comparing the affinities for oxygen of different hæmoglobins or at different temperatures. The value of our  $K$  is  $100/K_1$ ,  $K_1$  being the constant employed by Barcroft and Hill. The temperature coefficient is represented by the increase of the value of  $K$  with increase of temperature of  $10^\circ$ , or by the increase of the value of  $t_u$  since it is proportional to the equilibrium constant. The experimental error of the method employed involves an error of  $\pm 7$  p.c. in the value of  $K$ .

#### *Experiments on frog hæmoglobin.*

$pH=7.4$ ; blood diluted to such an extent, that the solution matches a dilution of human blood 1/40 (dilution of Hb about 1 : 280).

Frog 1.	Hb obtained from 1 ♀	( <i>Rana esculenta</i> ) no KCN
" 2.	" 2 ♀	1 drop of KCN (2 p.c.) to 20 c.c. of solution
" 3.	" 2 ♀	" " "
" 4.	" 1 ♀	No KCN " " "
" 5.	" 3 ♂	0.1 p.c. NaF
" 6.	" 2 ♀	"

We give as an illustration of our determinations the following table:

Frog 1, 15°.				
Pressure of O <sub>2</sub> mm. Hg	Per cent. saturation O <sub>2</sub> , corrected for presence of reduced Hb		Equilibrium constant K calculated from mean value of per cent. sat.	Mean value of K
	Observer:			
	J. B.	A. S.		
5.6	54	50	517	539
7.1	56	62	494	
9.3	60	62	595	
9.6	62	70	495	
18.8	75	77	594	

The values of K determined on samples of hæmoglobin from different frogs are given below.

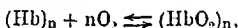
Frog	15°	25°	35°
1	539	—	—
2	663	1093	—
3	—	1330	5520
4	655	2189	—
5	975	2599	1189
6	502	1501	3357
Mean	661.7	1742.4	4355.1

*Experiments on human hæmoglobin.*

Blood from A.S., diluted 1:40, concentration of Hb 1:280; buffered pH=7.4, 0.1 p.c. NaF. Observed points in Fig. 3.

Temperature	15°	25°	35°
Value of K	24.25	141.5	762

The curves drawn from these data confirm the statement of Hartridge and Roughton, that the equilibrium between hæmoglobin and oxygen in dilute solutions is represented by a rectangular hyperbola, corresponding to the reaction



where  $n = 1$ .

Fig. 2 shows dissociation curves of frog Hb at 15°, 25° and 35°. For each temperature three curves are plotted, representing the highest,

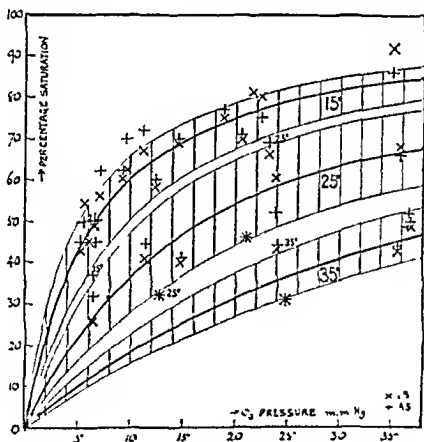


Fig. 2.

the lowest and the average mean value of  $K$ . Fig. 3 gives dissociation curves for human hæmoglobin. If we take the value of the unloading tension as a measure of the affinity of hæmoglobin for  $O_2$ , the affinity of frog blood appears to be much lower than that of human hæmoglobin. In order to get the same saturation of hæmoglobin, 50 p.c., the pressure of  $O_2$  must be at  $15^\circ$  0.2 mm. Hg for human Hb and 6.6 mm. for frog Hb, at  $25^\circ$  1 mm. and 17.4 mm. respectively, at  $35^\circ$  7.6 mm. and 43.6 mm. respectively. In other words, for obtaining the same effect in frog Hb,

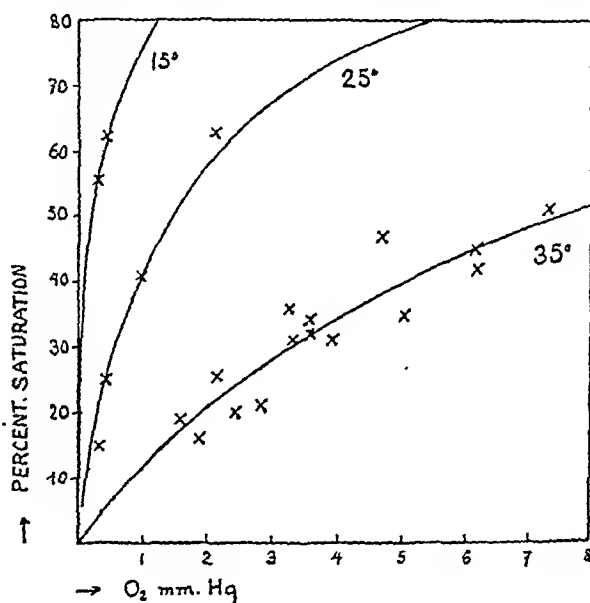


Fig. 3.

the pressure of  $O_2$ , compared with that sufficient for the 50 p.c. saturation of human Hb, must be raised at  $15^\circ$  32 times, at  $25^\circ$  12 times, at  $35^\circ$  6 times.

In Fig. 4 this comparison is made graphically for human and frog Hb at  $15^\circ$  and  $35^\circ$  and for *Planorbis* Hb at  $12^\circ$ . The dissociation of frog Hb at about  $20^\circ$  and human Hb at  $38^\circ$  could be represented by the same curve. One could assume, that these data may be in some relation with the optimal temperature for the physiological processes of the forms under consideration; but at present we must regard this relation as given by chance.

The temperature coefficient of the equilibrium between hæmoglobin and oxygen, calculated from the average value for  $K$ , is for frog Hb 2.6 for an increase of temperature from  $15^\circ$  to  $25^\circ$ , and 2.5 from  $25^\circ$  to  $35^\circ$  C. The same value 2.5, was found in our earlier experiments

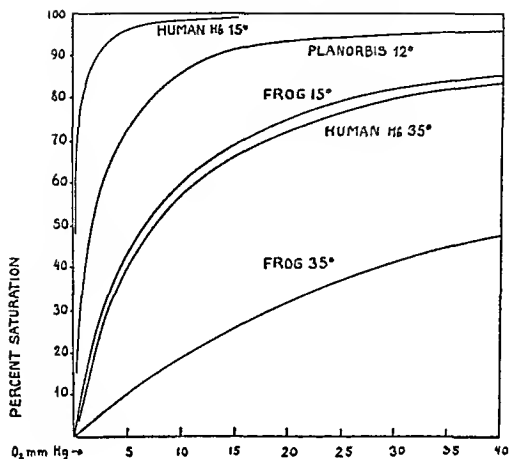


Fig. 4.

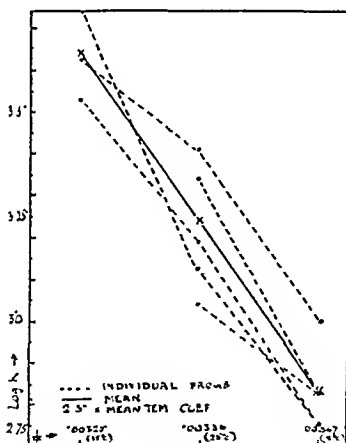


Fig. 5.

with the second method, over a range of temperature from 10° to 40° C.

In Fig. 5 the logarithms of the equilibrium constants are plotted against reciprocals of absolute temperature. The dotted lines represent values obtained from determinations on different samples of hæmoglobin, the thick line is drawn from the mean value. This relation is represented by a straight line, confirming the assumption, that the temperature coefficient is nearly constant over the range of temperature here considered.

From our experiments on human Hb we have calculated a temperature coefficient 5.8 from 15°–25° and 5.4 from 25° to 35°, mean value 5.6. This value is very close to that calculated from curves given by Barcroft and Hill(6) and Barcroft and Roberts(8) for more concentrated solutions of pure mammalian hæmoglobin, 5.4 over a range from 0° to 30°. We used for this comparison the figures, calculated by Krogh and Leitch(7). The equilibrium constants obtained by us are also not very different from those calculated from the data mentioned<sup>1</sup>.

Temp.	Equilibrium constant	
	Of human Hb calculated from our experiments	Of mammalian hæmoglobin, calculated from data by Barcroft and others
15°	24	22
25°	141	112
35°	721	700

In Fig. 6, the relation between temperature and equilibrium constant is represented for human hæmoglobin and compared with that of frog, tortoise and *Planorbis* hæmoglobin. Experiments on Hb solutions of the two last mentioned forms were made with the second method and therefore we cannot claim a great degree of accuracy for the absolute value of K. But the position of their equilibrium constant for a given temperature between those of frog and human Hb is quite certain, since we have done similar experiments on frog and human Hb, using the same method, and obtained not very different results. The relative affinity of a special Hb for oxygen can be read off from this figure by comparing the values of log K at a given temperature, this value being the greatest in forms with the lowest affinity for O<sub>2</sub>. The value of the temperature coefficient is represented graphically by the slope of lines.

Expressed in terms of thermodynamics, the value of the temperature

<sup>1</sup> We cannot compare our results on diluted Hb with those obtained by many experimenters on very concentrated solutions of Hb, since equilibria in these solutions do not obey the law deduced for the monomolecular reaction.



coefficient gains another significance, being no longer a mere proportional figure. It may be used in order to ascertain some physico-chemical

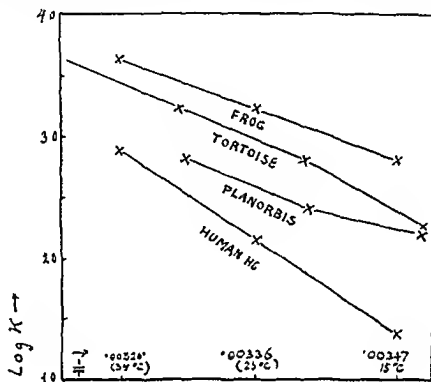


Fig. 6.

properties of the hæmoglobin molecule. Applying to the equilibrium between  $O_2$  and Hb van't Hoff's equation of the reaction isochore:

$$\frac{d \ln K}{dT} = -\frac{q}{RT^2},$$

where  $K$  is the equilibrium constant,  $T$  the absolute temperature,  $R$  the gas constant,  $q$  the heat produced by the reaction of one gram-molecule of hæmoglobin with oxygen, and, assuming that the value of  $q$  remains constant over the range of temperature considered in our experiments, and integrating between limits  $T_1$  and  $T_2$ , we get the equation

$$\ln K_2 - \ln K_1 = \frac{q}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right),$$

where  $K_1$  and  $K_2$  are the equilibrium constants at the absolute temperatures  $T_1$  and  $T_2$ . If  $R$  be 1.985 and ordinary logarithms be employed, the equation for the heat produced is (Nernst<sup>(9)</sup>)

$$q = -\frac{4.571 (\log K_2 - \log K_1) T_1 T_2}{T_2 - T_1} \text{ calories}$$

at the temperature  $\frac{T_1 + T_2}{2}$ .

The difference  $\log K_2 - \log K_1$  equals log of the temperature coefficient, when  $T_2 - T_1 = 10^\circ$  and when taking our equilibrium constants. But here a precaution is necessary. In the above formula we are dealing

with the concentrations and not with the partial pressures of the substances here considered, hæmoglobin and oxygen. In our case, when comparing equilibria at 50 p.c. saturation, the concentration of the hæmoglobin component is constant, as it can be easily deduced from the laws of mass action:

$$K_a [\text{Hb}][\text{O}_2] = K_b [\text{HbO}_2],$$

$$\frac{[\text{Hb}][\text{O}_2]}{[\text{HbO}_2]} = \frac{K_b}{K_a} = K,$$

at 50 p.c. saturation  $\text{Hb} = \text{HbO}_2$ , and the equilibrium constant in the above equation is directly proportional to the concentration of oxygen in the fluid, expressed in mols<sup>1</sup>.

Suppose the solubility of  $\text{O}_2$  in our solution of hæmoglobin to be the same as in pure water, we may calculate the molar concentration of  $\text{O}_2$  from the following data:

Temp. C°.	Solubility c.c. of $\text{O}_2$ per 1 c.c. of liquid	Molar concentration of $\text{O}_2$ at 1 mm. partial pressure of $\text{O}_2$ per c.c. mols	$\text{O}_2$ pressure in mm. Hg at half saturation		Molar concentration of dissolved $\text{O}_2$ at half saturation per c.c.	
			Frog Hb	Human Hb	Frog Hb	Human Hb
15	·034	$2 \cdot 10^{-3}$	6·6	0·24	$1 \cdot 33 \cdot 10^{-3}$	$4 \cdot 86 \cdot 10^{-10}$
25	·028	$1 \cdot 66 \cdot 10^{-3}$	17·4	1·4	$2 \cdot 89 \cdot 10^{-3}$	$2 \cdot 35 \cdot 10^{-9}$
35	·024	$1 \cdot 43 \cdot 10^{-3}$	43·6	7·6	$6 \cdot 24 \cdot 10^{-3}$	$1 \cdot 09 \cdot 10^{-8}$

Calculated from the above data, we found the value of  $q$  for human hæmoglobin to be 26·850 calories at 20° and 27·980 calories at 30°, and for frog hæmoglobin to be 13·290 calories at 20° and 13·980 calories at 30°. Barcroft and Hill have found for mammalian hæmoglobin  $q = 28,000$  calories.

The relation between  $q$ ,  $H$  heat given out by 1 gm. of hæmoglobin in union with  $\text{O}_2$ ,  $M$  molecular weight of Hb and  $n$  number of molecules of  $\text{O}_2$ , is represented by the formula

$$M = \frac{1}{n} \cdot \frac{q}{H}.$$

Since we obtained as dissociation curves rectangular hyperbolæ, the value of  $n$  may be put  $n = 1$ . Assuming the value for  $H = 1 \cdot 85$  cal. as found by Barcroft and Hill, the molecular weight of human Hb would be 15,000 and that of frog Hb 7300 approximately, or 1 m and 0·5 m respectively, where  $m$  may be about 16,000 or any manifold of that figure. However, the molecular weight of frog Hb may be the same as that of mammalian Hb, but then the value of  $H$  is much lower.

<sup>1</sup> If the temperature coefficient be calculated in this way, we obtain 4·7 for human Hb, and 2·2 for frog Hb.

Having no experimental evidence for ascertaining this value, we confine ourselves here only to showing the possibility of explaining differences in affinity for  $O_2$  between various hæmoglobins with differences in physico-chemical properties of the molecule.

The heat given out by the union of hæmoglobin with oxygen cannot be considered as of any biological significance, since the blood enters the lung capillaries comparatively highly oxygenated and, on the other hand, heat is absorbed, when  $O_2$  dissociates itself from Hb in the circulation. Moreover, the heat produced by union of Hb and  $O_2$  can be neglected altogether, when compared with the heat of evaporation in the lungs of mammals.

A few words may be said about the relation of the equilibrium constant to the "span," the difference in Å units between the position of maximum intensity for the  $\alpha$ -absorption bands of  $O_2$ Hb and that of COHb. If  $\log K$  at a given temperature be plotted against the length of span in Å units, the points representing this relationship fall nearly on a straight line in the case of human, hen, tortoise and frog hæmoglobin. But the points obtained from observations on *Planorbis* Hb fall on a very different place. A similar relationship between the equilibrium constant of the reaction



and the length of span holds good for a large number of vertebrates, as shown by Anson, Barcroft, Mirsky and Oinuma(2). As far as our observations go, no relation could be found between the position of  $\alpha$ -absorption band of  $O_2$ Hb in Å and the  $\log K$ .

*The significance of the affinity for  $O_2$  of different hæmoglobins.*

In the last few years a large amount of evidence has been put forward by different observers, working on various properties of hæmoglobin, in favour of the view, that hæmoglobin is not of invariable composition and that there are many differences in the properties of various hæmoglobins. We will not attempt to review these facts, since they were discussed quite recently by J. Barcroft and H. Barcroft(1), Anson, Barcroft, Mirsky and Oinuma(2), J. Barcroft(10), Anson and Mirsky(11), and Douglas, Haldane and Haldano(13); we may refer here for further information to their papers. Strong evidence was given for the view, that these differences are due to the globin portion of the molecule. We pointed out above, that there may be taken into consideration differences in heat given out by the reaction with  $O_2$  or possibly differences in molecular weight.

There have been some discussions on the adaptation of hæmoglobin to the biological conditions of various forms. Undoubtedly, such adaptations must exist, but at present we fail to see any decisive experimental evidence for this view, since we know very little about the gases of whole blood of other animals except mammals. Krogh and Leitch(7) have done some interesting experiments on the adaptation of the dissociation curves of blood of various fishes to their biological environment, but their conclusion, that the differences in curves are due to differences of the chemical environment of hæmoglobin inside the blood corpuscles, seem to us not to exclude another possibility of explanation. Krogh and Leitch supposed a uniformity of hæmoglobin and compared the actual dissociation curves of whole fish blood with the oxygen dissociation of mammalian hæmoglobin. We have not done experiments on fish hæmoglobin, but we conclude from the relationship between  $\log K$  and the length of span, taking the determinations published by Anson, Barcroft, Mirsky and Oinuma for two forms, carp and roach, that the affinity for  $O_2$  of hæmoglobin of those forms may be nearly the same as in frog.

Hæmoglobin itself is different in various forms of life. However, we do not know how this diversity of hæmoglobin influences the actual shape of the oxygen dissociation curves of whole blood in different animals. It would be certainly possible to apply the relation between the dissociation curves of mammalian hæmoglobin and mammalian whole blood to frog hæmoglobin, in order to construct the dissociation curve of frog's blood. But we are not convinced of the validity of such a proceeding. Moreover, the chemical environment of the hæmoglobin inside the blood corpuscles of cold-blooded vertebrates and birds, in other words, of the nucleated blood corpuscles, seems to be quite different from that of mammals. We may be perhaps allowed, to give in evidence of this view the following observations, which were made in this laboratory by Dr R. Weiss and ourselves, when attempting to obtain an information on blood gases of fowl, pigeon, tortoise and frog. In Barcroft's manometer a clotting of blood was observed, when a sample of frog's whole blood was shaken with ammonia. By adding potassium ferri-cyanide the blood sample was transformed very rapidly into a consistent jelly, which appeared to diminish its volume unceasingly, so that constant readings could not be obtained. Similar observations have been made by Krogh and Leitch on fish blood.

Brown and Hill(12), pointing out the considerable interest which the effect of temperature on the dissociation curve of blood has in

general physiology, especially in relation to cold-blooded animals, drew attention to the significance of the lowering of the unloading tension with decrease of temperature for the rate of the recovery process in muscle. This is very much slowed by a fall of temperature. Since hereby the oxygen supply to the muscle would be reduced also, the recovery process in cold-blooded animals would be very prolonged. If we apply our results to this problem, the conditions for recovery, as far as the reduction of oxygen pressure in frog's muscle is concerned, seems to us to be not as bad as one would conclude when taking data for the absolute value of the unloading tension of  $O_2$  in mammalian Hb. Taking into account only the function of Hb and supposing that all other conditions be identical, the conditions of recovery in frog's muscle at  $15^\circ$  may be about the same as those in the muscles of a mammal at  $35^\circ$ . But we must regard at present such considerations as being only of a problematical value; they cannot be taken as proofs for an adaptation of hæmoglobin to the biological conditions of cold-blooded animals.

#### SUMMARY.

1. Oxygen dissociation curves of diluted solutions of frog and human hæmoglobin, concentration of Hb 1 : 280 and 1 : 1500,  $pH = 7.4$ , were determined with Hartridge reversion spectroscope at  $15^\circ$ ,  $25^\circ$  and  $35^\circ$  at no  $CO_2$  tension. Some additional observations were made on fowl, tortoise and *Planorbis* hæmoglobin.

2. Dissociation curves of such solutions of hæmoglobin, which were not free from salts, are represented by rectangular hyperbolæ. Those of human Hb are about of the same order as obtained by Barcroft and co-workers on more concentrated solutions of pure mammalian hæmoglobin.

3. The affinity of frog hæmoglobin for oxygen is much lower than that of human hæmoglobin. The value  $t_u$ , Krogh's tension of unloading, is for human Hb 0.2 mm. Hg at  $15^\circ$ , 1.15 mm. at  $25^\circ$ , 7.5 mm. at  $35^\circ$ , for frog Hb 6.5 mm. at  $15^\circ$ , 17 mm. at  $25^\circ$ , 44 mm. at  $35^\circ$  C.

4. The value of the equilibrium constant and the value of the tension of unloading, which is proportional to the constant, increases for an increase of  $10^\circ$  2.5 times in frog hæmoglobin and 5.6 times in human hæmoglobin over a range of temperature from  $15^\circ$  to  $35^\circ$  C.

5. The heat of reaction between one gram-molecule of hæmoglobin and  $O_2$  is 27.400 calories in human hæmoglobin and 13.600 calories in frog hæmoglobin.

We wish to express our thanks to Prof. Langley for granting us the facilities of the laboratory and to Mr J. Barcroft for his advice and his great help during the course of our experiments.

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A CONTRIBUTION TO THE PHYSIOLOGY OF THE  
SPLEEN. BY J. BARCROFT, H. A. HARRIS,  
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A NUMBER of researches carried out in the Cambridge laboratory have pointed to the conclusion that the spleen has a very definite function which is, after all, but the expression of the two chief features in the structure, namely the muscular character of its framework and the extravascular condition of the red blood corpuscles which it contains. This function may be described briefly as that of acting as a reservoir for erythrocytes. J and H. Barcroft(1) elaborated a technique for comparing the percentage of CO hæmoglobin in the spleen with that in the general circulation under known conditions of carbon monoxide inhalation and showed that there was a much greater lag in the rate at which CO entered the hæmoglobin of the spleen, than that of other organs. This presumably was due to the fact that the blood was outside the general circulation and indicated that the separation was functionally much more complete than was usually supposed. Hanak and Harkavy(2) expanded these experiments and showed that in animals which were at rest, two 'hours' respiration of concentrations of CO sufficient to raise the COHb in the blood to 20 p.c. might elapse before any CO appeared in the spleen pulp. In such cases there could be no actual circulation through the parenchyma of the pulp, the blood was therefore held in a reservoir. This observation yielded no information as to whether the reservoir were large or small, important or unimportant. Hanak and Harkavy did show, however, that the reservoir was evacuated on struggling, a fact which suggested its functional nature.

These facts together with others to be referred to later on the effect of temperature pointed to the spleen expelling its corpuscles under such circumstances as increased the demand on the blood relatively to the quantity of functional hæmoglobin in circulation. There were however

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certain difficulties about this view. The first difficulty was the current idea that possession of a spleen "makes no difference." Barcroft, Murray, Orahovats, Sands and Weiss(3), however, showed that guinea-pigs from which the spleen had been excised died sooner in an atmosphere charged with coal gas than did normal guinea-pigs or operated controls from which a portion of pancreas, or omentum, or one horn of the uterus had been excised; whilst in hydrocyanic gas, the lethal nature of which is quite unconnected with the carriage of oxygen by hæmoglobin, the normal animals enjoyed no greater tenure of life than the splenectomised ones. Faced therefore with such an emergency as we conceived it to be the purpose of the spleen to meet, the splenectomised animals were at an evident disadvantage. The second difficulty about our view lay in the size of the spleen, which appeared to be too small to admit of any very significant mass of corpuscles being evacuated from its substance. On looking into this matter it appeared that no knowledge was forthcoming as to what size the spleen actually was in the normal body, untouched by operation.

Since contraction of the spleen follows on anoxæmia of the central nervous system as shown by de Boer and Carroll(4) almost any form of death might be expected to be accompanied by contraction of the spleen—certainly any death which was caused by a general anoxæmia, *e.g.* hæmorrhage, carbon monoxide poisoning, etc. Further, if our view is correct, it should be possible to demonstrate a considerable contraction of the spleen on exercise, for exercise would be the most obvious condition, in the ordinary circumstances of life in which an increased demand is made on the supply of oxygen-carrying hæmoglobin.

The present paper describes experiments, designed partly to ascertain whether there is a sufficiently definite relation between the size of what may be considered as the resting spleen in life to that after death, to allow of the size after death to be used as a measure to the resting size during life, and whether the spleen during rest might on occasions be much larger than would be inferred from its size in the dead animal. Secondly some experiments were made on the effect of exercise on the volume of the spleen during life and a comparison was drawn between the size of the organ under conditions of exercise or impaired oxygen supply with those of the resting and the dead spleens respectively. These points form the preliminary to a more general discussion of the spleen regarded as a regulator of the blood volume.

In order to discover the size of the spleen in the normal living body two techniques seem to be possible, (1) to replace a portion of the body



wall with a celluloid window<sup>1</sup>, (2) to resort to radiography. The experiments described in the present paper were all carried out with X-rays. We would like here to thank Dr Dale for a suggestion as to the actual X-ray technique which we adopted and which has proved quite satisfactory, namely that of performing a preliminary operation in which a number of metal indices are placed round the edge of the spleen. The animal is then allowed to recover from the operation and photos have been taken usually about a week afterwards. The animal makes a complete recovery. The health of the spleen does not appear to suffer; we have one rabbit which was operated upon more than six months ago and which has shown no ill effect from the abnormal metallic contents of its abdomen. The actual indices which we used were surgeons' metallic sutures. These are easily placed on the spleen and can be filed beforehand into distinctive shapes, if necessary. The preliminary operation was carried out under chloroform and ether anaesthesia.

*Preliminary experiments* were carried out upon rabbits. These animals frequently have spleens which are rather long in relation to their breadth, and become almost cord-like when contracted. At first we put but two sutures into the spleen, one at each end. When the rabbit was placed on the X-ray table either on its side or on its stomach the two sutures could easily be seen on the fluorescent screen. Moreover they were seen invariably to approximate either after exercise, or when blood was withdrawn from the jugular vein. These experiments were of course of doubtful interpretation inasmuch as in order to obtain views in perpendicular planes of space the rabbits were rotated, nevertheless the alteration in the position of the sutures was very remarkable and convinced us of the desirability of carrying out more exact experiments.

*Technique.* Our final technique was as follows. Cats were used for the most part; rabbits, monkeys and one dog being used for confirmatory tests. Usually five, six, or seven sutures were put round the edge of the spleen. The operations were carried out in the Cambridge Physiological Laboratory, when the wound had been healed they were taken up to the X-ray department in the Anatomical Laboratory at University College, London, there to be photographed. The installation gives good records with an eighth of a second exposure. Records can be taken from the lateral and dorsal aspects within 10 seconds of one another. Care was taken in every case to discard all results in which the animal was

<sup>1</sup> Since the above was written by the kindness of Dr Flörke who had such a cat, I have seen the spleen contract when the cat took exercise.

seen to have moved between the two exposures. In our earlier experiments the cats were placed in a cardboard box. Later we held them in position, the fore legs in one hand and the hind legs in the other.

The records were treated in the following way. Prints were made from the films. The print of the lateral aspect was laid on a block of paraffin with a level horizontal surface. The position where the teeth of the suture met was marked in the case of each suture, a sewing needle was driven vertically through the mark, so that a needle marking the position of each suture stood up from the block, perpendicularly to its surface. The corresponding sutures were identified and marked on the dorsal view, a line was drawn on the print either through or parallel to the middle line of the spinal cord, the distance of each suture was measured from this line. The needle corresponding to that suture was then driven into the block until the distance of its head from the paraffin surface was the same as that of the suture from the line on the antero-posterior print. The heads of the needles then gave a slightly magnified but relatively correct register of the positions of the sutures in space. The magnification was the same for all photos of the same animal. A thread was tied into the eye of each needle and a copper wire was fitted to the heads and tied in place, making a figure which represented the edge of the spleen.

In obtaining this wire figure due regard had to be paid to the orientation of the sutures for in some cases the spleen was more than usually curved, and in one or two there was obviously a considerable change in the dimensions of the spleen without very much alteration in the positions of the sutures. A simplified example may explain. Thus if *A* and *B* are two needles, they might correspond to sutures the same distance apart in two successive photos of the spleen, yet the aspects of which might be as in Figs. 1 and 2 respectively, in which case the sutures would indicate spleens of very different configuration.

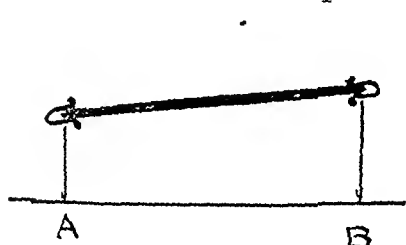


Fig. 1.

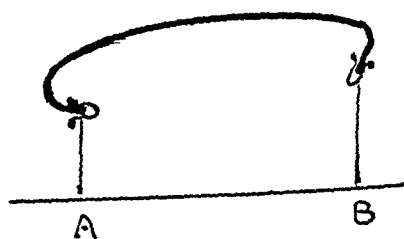


Fig. 2.

The wire figure is tied across in sufficient places in order to insure its retaining its shape, the threads are then cut and the figure removed.

Sheet lead of standard thickness is fitted to it, the figure marked on the lead, and a lead figure is cut which when flattened represents as a rough approximation the surface which the spleen would cover if taken out and laid flat on the table. As we became more expert it was possible in the simpler cases to leave out the step of making the wire figures and fit the lead directly to the heads of the needles. In a few cases thick paper was used instead of lead.

The method indicated above is of course a very crude one and it would be quite incapable of dealing with small changes in the size of the organ. The following are some examples of instances in which duplicate lead models were made independently by different persons from the same pair of films.

Orahovats	32.9	23.1	21.7	28.0
Barcroft	34.3	28.2	23.0	28.7

Barcroft's results, which were made from the wire tend to be larger than Orahovats's, in which the lead is fitted directly to the top of the needles. The wire models tend to give a more rounded figure. The ratio of the weights of any pair of models made by a single method is as a rule less affected than the individual models.

So far we have dealt only with two planes. There seems to be no reason, judging from the histology of the spleen, why a general contraction should affect the third plane differently from the other two and therefore one may get an idea of the actual percentage change in the volume of the organ by taking as a basis for comparison, the cube of the square root of the weight of the lead model. If one of the photos taken in any series be that of the spleen in the dead animal and if the weight of the spleen *post mortem* be known, a computation may be made on the lines indicated above, of the weights of the spleens previously photographed under the other conditions.

Some notion of the accuracy of the method may be obtained by taking two pairs of photos of the spleen in the body, *post mortem*, and comparing the models obtained from them. In such a case the separate estimates of



Fig 3 Photos of lead models reconstructed from the same photos. A, without wire; B, with wire weighing respectively 28.0 and 28.7 gm.

the spleen made from photos taken an hour after death, and the day after, but in both cases before the body was opened gave 6.6 and 6.5 gm. respectively.

*The resting spleen.*

In the living animal a greater difference may be expected in successive computations of the volume of the spleen, for the spleen itself may vary somewhat from time to time even under nearly similar conditions, owing to a greater or less degree of tone in its muscle. Its shape and position also will be influenced by the degree of distension of adjacent organs such as the stomach. The following are some such computations:

Animal	...	...	...	Cat				Monkey	Dog
		(1)	(2)	(3)	(4)	(1)	(1)		
Weight of spleen (gm.)	<i>a</i>	26.4	19.8	40.5	32.9	7.5	66		
	<i>b</i>	24.1	22.7	44	28	6.9	75		
	<i>c</i>	—	—	—	—	—	60		

From the above instances it appears that the computations of the spleen in the resting animal differ by about 20 p.c. of the maximum measurement. Fig. 6, p. 454, shows four photographs of lead models of the spleen of the dog cited above when at rest. Of these *C*, *A* and *D* form the basis of the determinations given above (*a*, *b* and *c* respectively).

What size then is the spleen in the resting cat? The limits according to our observations are very wide. In full grown cats from about 2 kilos upwards (1990 gm.) nineteen estimations were made; the smallest spleen appeared as 11.9 gm. and the largest 45 gm., nine were between 14 and 20 gm., four were between 20 and 30 gm. and four were over 30 gm. The average is 21 gm., the median 18 gm. One may picture the spleen in the resting cat therefore as being of the order of 20 gm. in size with the possibility of large variations in different animals. The extent to which this size is correlated with definite factors such as age has not been worked out.

In fourteen of these cases we had the weights of the cats which average 2.9 kilos and the median 2.7. The weight of the spleen then appears to be about .7 p.c. of the weight of the cat in good sized adults. In two monkeys it appeared to be definitely smaller; they were 7.5 gm. and 5.7 in monkeys of 4.6 (old) and 2.0 (young) kilos respectively. In dogs only one experiment was performed; in this the spleen weighed 1.5 p.c. of the weight of the dog.

*Relation of the living to the dead spleen.*

In all cases of cats the spleen in the cadaver was lighter than the organ as computed in life. So far as our observation went the difference depended to some extent on the way in which the cats were killed.

*Histamine.* The least change, somewhat to our surprise, was in the case of cats killed by histaminic.

Serial number	W Weight of cat (kilos)	A Weight of spleen in resting cat (grams)	B Weight of spleen in dead cat (grams)	D Difference (grams)
5	36 0	12.3	9 8	4
6	28 5	17 8	13 0	4 8

*Coal gas poisoning.* Two series of experiments were carried out in coal gas poisoning.

In the first series the concentration of gas was great and death was sudden, taking place in  $1\frac{1}{2}$  to 4 minutes, in most cases about two. In the second series, which consists of three experiments, the poisoning was more gradual, in all cases the spleen contracted on death, the degree of contraction however differed greatly in different animals. The data are as follows the headings being as in the table above:

Series Cat	W (kilos)	A (grams)	B (grams)	D (grams)
I. 7	2.4	14.3	11.7	2.6
8	2.7	14.0	12.1	1.9
9	3.1	34.0	19.7	14.3
10	2.0	22.1	12.2	11.9
11	—	11.9	8.0	3.9
12	—	17.4	8.0	9.4
III. 13	2.5	17.9	12.5	5.4
14	2.4	14.8	10.0	4.8
15	2.0	15.4	13.0	2.4

Among the spleens which contracted least was the last. The experiment deserves a word of comment as shedding some light on the variableness of the results. As will be shown later, exercise is one cause of contraction of the spleen. The cat in question greatly resented being photographed and it proved impossible to obtain records without holding her in position by main force. It is probable therefore that the spleen of the imprisoned cat was considerably contracted. The inconsiderable amount of contraction in some of the earlier experiments may have had to do with the suddenness of the poisoning; we have no real knowledge of the time relations of the contraction in the intact animal. The important thing to notice is that some of the spleens squeezed out 9-15 c.c. of their contents into the circulation.

*Drowning.* Four experiments were carried out in which the cat was drowned by tying a weight round its neck and placing it in the sink. They gave the following result:

Cat	W (kilos)	A (grams)	B (grams)	D (grams)
16	4.4	45.1	32.2	12.9
17	3.5	16.7	9.4	7.3
18	—	20.5	10.1	10.5
19	—	30.5	12.1	18.4

Drowning combines muscular exertion and anoxæmia. It is not surprising therefore that the shrinkage of the organ is very great, amounting in one case to 18.4 c.c.

*Hæmorrhage.* The form of death which in cats gave the most striking results was hæmorrhage. The subject may be dealt with in somewhat greater detail than the other forms of death which have been considered. As a sample experiment the following may be taken; a cat 2.9 kilos in weight, was photographed twice at rest, it was then given urethane and photographed again, in this case with little change in the size of the spleen. Under urethane the spleen was computed as weighing 28.3 gm., before the urethane 32.9 and 28.1 gm. respectively. On one occasion out of four we obtained a noticeable contraction in the "urethane" animal. The cat was then subjected to successive bleedings at intervals of about 10-15 minutes. The extent of the bleedings may be seen from the following table.

Cat 4	Time	Blood lost	A Relative weight of model	B Relative weight spleens = $\frac{3}{\sqrt{A}}$	Weight of spleen $B \times 10/36.2$ (grams)
Rest 1	—	—	24.5	124	34.3
Rest 2	—	—	21.9	102	28.2
Urethane	2.50	—	22.0	103	28.5
1st bleeding	3.20	10	16.4	66.7	18.7
2nd "	3.30	22	14.1	53.0	14.6
3rd "	3.40	36	—	—	—
4th "	3.53	41	14.5	55.1	15.2
5th "	4.10	71	13.8	51.3	14.2
6th "	4.28	83	12.1	42.2	11.6
7th "	4.40	95	13.1	47.5	13.1
8th "	4.53	100	9.9	31.4	8.7
P.M.	—	—	11.0	36.2	10

Weight of spleen P.M. = 10 gm.

Weight of cat 2.87 kilos.

It may be interesting to give the relative weights of the living spleen, the spleen at death and the *post mortem* spleen in the four cases of hæmorrhage which we have studied.

Cat	A Weight of spleen P.M. (gm.)	B Weight of spleen at death (gm.)	C Weight of spleen at rest (gm.)	Ratio C/A	Ratio C/B
1	6.6	6.7	25.3*	3.8 : 1	3.7 : 1
2	6.6	6.5	19.8	3 : 1	3.1 : 1
3	17.5	13.6	42	2.4 : 1	3.1 : 1
4	10	8.5	30.5	3.1 : 1	3.6 : 1
Dog	13.3	13.3	66	5.0 : 1	5.0 : 1

\* Average of two determinations.

To us it came as a great surprise, that the *post mortem* spleen gave so paltry an idea of the spleen in the living animal: if there is so great a shrinkage one may ask, what actual decrease is there in the volume of the blood during hæmorrhage? Some idea of this may be obtained by comparing the volume of the blood lost in hæmorrhage with that added by the contraction of the spleen. Thus in Cat 4, 10 c.c. of blood were withdrawn, the spleen weight decreased by 9.8 gm. The volume of blood, therefore, was not appreciably altered by the hæmorrhage. Fig. 4 shows the extent to which hæmorrhage was balanced by contraction of the spleen in Cat 4. The broken line showing the volume of blood withdrawn, naturally goes diagonally across the figure; the contribution made by

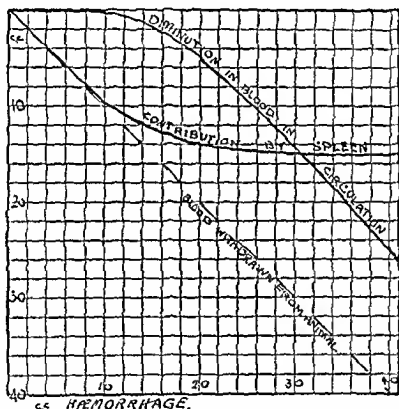


Fig. 4. Effect of hæmorrhage on spleen volume and blood volume. If the blood volume be taken as 150 c.c. the figures in the ordinate  $\times \frac{2}{3}$  represent percentages of the blood volume.

the spleen at first so nearly coincides with this line, that when the blood contributed is added to the blood lost the quantity of circulating fluid scarcely changes.

Comment may be made on two points which are not unconnected.

(1) The spleen gives up the principal mass of blood at the commencement of the hæmorrhage. This is of course no measure of the actual force of contraction which at the end may be as great as, or greater than, at the commencement of the period of contraction.

(2) In the case of the cat one fact has come out pretty consistently, namely that over the first ten cubic centimetres of hæmorrhage, the blood volume appears to undergo little or no sensible diminution, i.e. the amount of fluid added by the spleen is almost the same as that lost in the hæmorrhage. A cat of course can lose very much more than ten cubic centimetres of blood, yet a hæmorrhage of that degree would be a very large one in the ordinary accidents of feline life; it would amount to about one-fifteenth of the whole blood volume of the animal and would therefore correspond to about 300 c.c. in man.

In the one dog which we investigated the variations in the size of the spleen were proportionately greater than in the cat, for the resting spleen at its largest was more than five times the weight of the *post mortem* spleen. The animal was bled to death under urethane, the main features being much as in the cat though on a larger scale. Here again the greatest loss from the spleen was at the earlier part of the hæmorrhage, though not quite so early as in the cat; the figures are as follows:

Hæmorrhage	(1)	(2)	(3)	(4)	(5)	(6)
Total quantity of blood lost by dog	20	40	68	133	200	230
Total quantity of blood lost by spleen	5	25	39	44	50	52

The dog being 4.45 kilos, the blood volume at rest was of the order of 300 c.c. The hæmorrhage extended over two hours. We need not here enter into the discussion as to the ratio of the blood volume to the body weight; the blood volume by some observers has been placed as low as 1/20 of the body volume, which for this dog would be 223 c.c. or rather less than the actual amount of blood which was obtained from the dog. We mention the matter here because it illustrates in a striking way the fact that much still remains to be discovered as to the rate at which blood is made during a hæmorrhage which lasts over two hours and we hope to undertake work on this subject.

The principal interest in the foregoing pages lies in the light which they throw on the general thesis, that the spleen has as one of its functions the adjustment of the volume of circulating blood to the needs of the animal. Our general conception is that while the majority of the red blood corpuscles are circulating, as "currency" a fraction of them are held in reserve, "banked" so to speak, and that one "bank" is the spleen.

So far as exercise is concerned we have performed experiments on the dog, the cat and the rabbit. The method has been that described above. In preliminary experiments on the rabbit the animal was laid on the X-ray table, the distance of sutures at the two ends of the spleen



was measured, the rabbit was then encouraged to "kick about," as rabbits do, and a second measurement taken, there was no difficulty

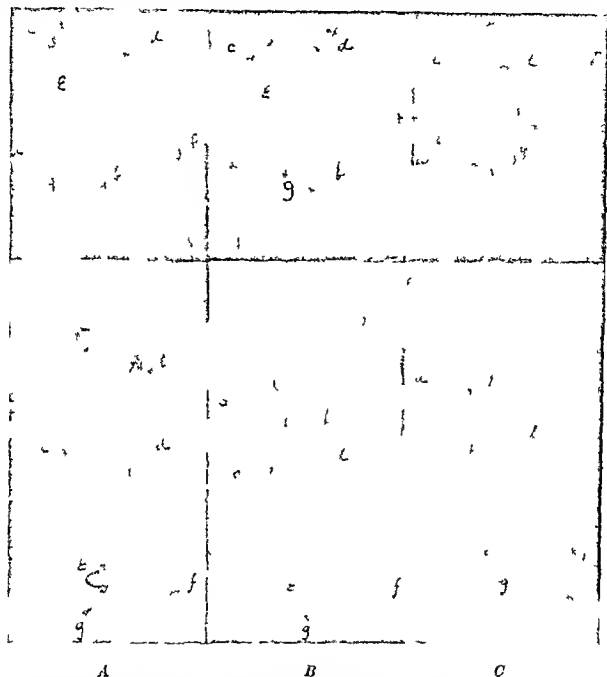


Fig 5  $\times \frac{2}{3}$  Photos showing the clips in the margin of the spleen of a dog *A* at rest, *B* after severe exercise, *C* after severe hæmorrhage. Upper photos taken in antero-posterior plane. Lower photos in the later plane. The photos of the lead models are shown in Fig 7 *C* and *A* respectively.

in demonstrating that in whatever position the measurement was made, the sutures were nearer together after the exercise than before it. These preliminary experiments have been entirely confirmed on the dog, the cat and the rabbit by the reconstruction method.

In the dog two sets of photos were taken at rest which gave the spleen a weight of 68 and 75 gm respectively (Fig 6 *A* and *D*). The dog

then ran about, but the exercise was not so great as to make it perceptibly out of breath; photos at this stage showed the spleen to have a weight of

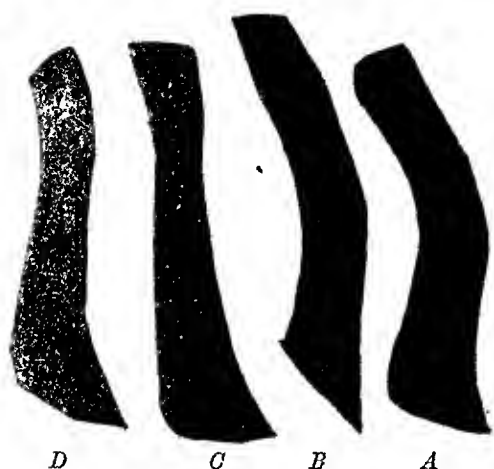


Fig. 6. Photos of four lead models of resting spleen of the same dog observed on different occasions (*B* under urethane). The actual length of *B* was 11.8 cm.



Fig. 7. *A* as in Fig. 6; *B* after severe exercise, *C* after severe hæmorrhage.

35 gm.; it was then exercised more vigorously so that at the end it was panting and its tongue was hanging out. The spleen had shrunk to 28 gm. In round numbers after mild exercise the spleen expelled half its substance, after severe exercise two-thirds. The actual quantity of material which left the organ as between the largest resting measurement and that after mild exercise was 40 c.c. whilst 8 more c.c. were expelled as the result of severe exertion. Taking the blood volume of this dog as 300 c.c. the amount contributed by contraction of the spleen was 15 p.c. of the blood volume. In this experiment there is no reason to suppose that any factors need be considered other than those of purely natural exercise. It is more difficult to induce the cat to take violent exercise without the possibility of inducing some degree of apprehension which would cause an adrenalin secretion and we know that adrenalin causes contraction of the spleen, but on the other hand it must be remembered that in the course of nature the same may be said; fear is the usual incentive to considerable exercise in a cat. In the first three of the four experiments which are cited below the cat was chased about the room in a very moderate way, in the fourth it was made to fight by placing it standing on a sheet which was then lifted by the corners. The cat finding itself on an unsteady surface became extremely active. In all cases the spleen contracted.

Cat	Weight of spleen (grams)		C, weight of material lost by spleen (grams)	Approximate proportion of C to blood volume taken as 150 c c
	A, before exercise	B, after exercise		
1	26.4	13.7	12.7	85 %
	24.1	7.1	17.9	12
2	19.8	9.9	9.9	66

In the rabbit the degree of shrinkage, expressed as a proportion of the size of the spleen, is of the same order as in the cat and dog, but the absolute amount of material expelled is less proportionately to the blood volume for the spleen is a smaller, and often a very much smaller, organ relatively

Rabbit	Relative weight of the spleen before and after exercise	
	Before	After
1	100	56
2	100	60

In the two monkeys at our disposal we found it impossible to induce exercise uncomplicated by rage, possibly such could be accomplished on a tread mill, but it must at once be confessed that the spleens of these monkeys were so small that a 50 per cent contraction would add but little to the blood volume, we never found it possible to photograph the spleens unless the monkeys were under the influence of a narcotic. Moreover the monkey's spleen offered special difficulties on account of its shape. As we saw it, it approximated to a tetrahedron, each facet of which was an equilateral triangle. This figure could easily have changed into a sphere without any alteration in the positions of the sutures at its corners. The alterations in volume of the monkey's spleen and therefore its volume when at rest may then have been much greater than we suppose.

Summing up what we have said above, it would seem that during strong exercise the spleen may expel more than half its volume of blood and shrinks to about the size of the spleen after death. We conclude that the *post mortem* volume of the spleen may be taken as a minimum estimate of the volume of blood it drives into the circulation during strong exercise. On this basis the spleen in man would expel from 110-258 c c of blood. If, as is possible, some concentration of blood takes place in the spleen pulp, the volume of red corpuscles passed out will be greater than corresponds to the decrease in spleen volume taken as blood. We arrive then at the general conclusion that the importance of the spleen as a reservoir of blood depends upon its size. The ratio of *post mortem* weight of spleen to body weight varies, as is known, in

different animals. The data seem to us to suggest that the ratio is greater, the greater the normal activity of the animal.

In the animals we observed the ratio was as follows: In the two monkeys it was  $\frac{1}{33.3}$  and  $\frac{1}{70.0}$ . The extreme variation in cats was  $\frac{1}{13.7}$  and  $\frac{1}{7.0}$ , but in eight cats it only varied between  $\frac{1}{20.0}$  and  $\frac{1}{30.0}$ . In the one dog experimented on, the ratio was  $\frac{1}{33.0}$ .

#### SUMMARY.

1. A method is described for the computation of the size of the spleen in the dog, cat, rabbit and monkey, during life without the necessity of an anæsthetic or an operation synchronously.

2. The normal spleen is usually much larger than that of the dead animal. The forms of death studied were in most cases such as induced one or other type of general anoxæmia, *e.g.* CO poisoning, drowning, hæmorrhage.

3. In the case of hæmorrhage the living spleen in the dog, cat or rabbit is two, three or even five times the size of that of the dead animal.

4. In the earlier stages of hæmorrhage (in the cat up to 10 c.c. or 8 p.c. of the blood volume) the spleen contributes an amount of material to the circulating blood approximately equal to that of which the hæmorrhage deprives it. In the dog also the principal evacuation is at the commencement of the hæmorrhage.

5. During exercise also, the spleen expels its contents into the circulation in a great degree. Estimates in the cat and dog show that in exercise the shrinkage of the spleen corresponds to 6-15 p.c. of the blood volume.

6. The view is put forward that the spleen exercises a real function in adjusting the volume of circulating blood, or more correctly circulating functional hæmoglobin according to the needs of the animal.

We have to thank the Medical Research Council, and the Royal Society for grants parts of which have been used for this research and the Rockefeller Foundation which defrayed the cost of the monkeys. We should like also to say a word of appreciation of Mr Melville, technical assistant to the X-ray department at University College.

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STUDIES ON THE INNERVATION OF SMOOTH MUSCLE. III. Splanchnic Effects on the Lower End of the Oesophagus and Stomach of the Cat. BY HARRY O. VEACH.

*(From the Physiological Laboratories, Cambridge, and the Harvard Medical School.)*

IN the first article of this series (1, p. 258), it was stated that evidence in favour of an explanation advanced therein for inhibition of parts of the alimentary tract by the vagus had been obtained by stimulation of fibres coursing from the coeliac ganglion. Further investigation of the problem, however, has led to the conclusion that the splanchnic exercises its inhibitory action on the stomach by a method different from that employed by the vagus, though the results tend to support the conclusions reached for vagal inhibition. In the continuation of the investigation, the splanchnic major nerve, rather than fibres peripheral to the coeliac ganglion, has been subjected to faradisation. The method was modified thus, because Dr J. R. Pereira and the author (2) have found that the frequency of discharge from the cells of the superior cervical ganglion, and probably, therefore, from other peripheral nerve cells, is directly proportional to the frequency of impulses delivered to them from the preganglionic fibres.

*Methods.* The experimental method employed at Harvard has been described quite fully in a preceding paper (1), and the method at Cambridge was much the same. Shocks delivered by induction coils, whose primaries were supplied with iron cores, were used for stimulation. The primary current was supplied in all cases by a 2-volt accumulator, its intensity for the splanchnics, in the Cambridge experiments, being adjusted to .09 ampère. In these experiments, moreover, the same inductorium, together with the new rotary interrupter described previously (2), was used exclusively for the splanchnics. At a secondary position of 8 cm., the shocks became detectable by the tongue. At 5 cm. they were weak; at 3 cm. they were moderately strong, and at 0 cm. they were slightly painful to the tongue, though they could be readily borne on it. In giving secondary positions in the following account of the results, the reference is to this inductorium. A rotary interrupter previously described (1) was employed also for the splanchnics at Harvard.

The method in which these interrupters were used, and the arrangements in the secondary circuits, did not differ from that described in the papers referred to. When both vagus and splanchnic were stimulated simultaneously, the series of cam wheels, *E*, of the Lucas interrupter, described by Adrian(3), served as contact breaker for the inductorium used in stimulating the vagus. The method in which this device was used has been described previously(2).

Platinum or silver electrodes were used in stimulation. The end of each wire, in the later experiments, was bent into a hook, the two hooks pointing in opposite directions, to prevent slipping off of the nerve. The electrodes were supplied usually with glass shields, though infrequently a rubber tube shield, similar to that described by Cannon(4), was employed. The latter type of shield was used almost exclusively in stimulating the fibre bundle peripheral to the coeliac ganglion. The glass shield was modified somewhat from that described by Sherrington(5), to eliminate the stopper at one end. Both ends of the shield were of a similar conical shape, the string connecting with the nerve being drawn out through the small opening and tied to the insulated leads to the electrodes. The total length of the glass shields varied from 2.5 to 3 cm.

In all experiments, the isolated vagus nerves, or at times the vago-sympathetic trunks, were cut in the neck. The splanchnic nerve was stimulated either above or below the diaphragm. In the former case, the sympathetic chain was isolated for about 2 cm. above the origin of the nerve through an incision between two of the lowermost ribs. The chain was cut at the cephalic end of this isolated portion; the rami communicantes were cut caudal to the section, and the splanchnic was then isolated almost to its exit through the diaphragm. Occasionally a smaller nerve appeared to emerge from the chain a few mm. below the splanchnic major, and an attempt was made to draw it with the splanchnic between the electrodes. This was probably the first minor. Once the splanchnic major was apparently divided into two separate trunks. Frequently the continuation of the chain beyond the splanchnic was cut. For stimulation beneath the diaphragm, the nerve was isolated through the incision for removal of the corresponding adrenal gland. The diaphragm was separated from its attachment to the lateral body wall, and the splanchnic major was isolated up to its connection with the sympathetic chain. Several mm. of the chain cephalad of the origin of the nerve were isolated also and left attached to the splanchnic. The rami communicantes and the continuation of the chain caudad were cut, and the nerve was brought beneath the diaphragm. A preparation

3 cm. long could be made thus before the coeliac ganglion was reached. The adrenal gland on each side was usually removed through an incision about 1 cm. beneath the last rib. In this operation, the portion of peritoneum overlying the adrenal was removed also, this being the only opening made into the abdominal cavity. In four experiments, however, the adrenals were removed through a median line incision through the linea alba, the incision being closed with sutures or covered with flannel moist with Ringer solution. In these cases, the viscera were considered exposed, but the results did not differ from those obtained when the adrenals were removed from the back.

The splanchnic major was stimulated in 38 cats. In most cases, both splanchnic majors, and in a large proportion, both adrenal glands were removed before the termination of the experiments. In eleven of the later experiments, the animals were prepared at the outset by cutting the vagi and both splanchnic majors, and removing both adrenals. As a rule, the animals were anaesthetised at first with ether or chloroform, or a mixture of both, and a dose of 3.5 to 5.0 c.c. of saturated chloralose solution in Ringer (a concentration of somewhat less than 1 p.c. of the drug) was injected intravenously. Ether was used thereafter if required. Two of the animals, however, were anaesthetised with urethane, two with ether, and two were decerebrated. Artificial respiration was administered by tracheal insufflation. Arterial blood-pressure was taken with an ordinary mercury manometer. In a few experiments the anti-coagulant was concentrated sodium carbonate, but usually it was 4 p.c. sodium citrate. Special procedures will be described in connection with the results obtained.

*Stimulation of fibres peripheral to the coeliac ganglion.*

The observations on stimulation of fibres peripheral to the coeliac ganglion were relatively few in number. Only motor effects were produced on the lower end of the oesophagus, the vagi and splanchnics having been cut, but often this part of the canal was apparently unaffected. The motor reaction consisted of a more or less marked and well maintained tonic contraction, on which rhythmic contractions were superposed, and it usually disappeared gradually on cessation of stimulation. In the case of the stomach, however, both inhibition and contraction occurred, though the former was probably more common. The occurrence of contraction was apparently favoured by a relatively low frequency of stimulation. In the one experiment in which a simultaneous record of blood-pressure was taken, a frequency of 15 per second

(475 z units) caused relaxation, whereas 5 per second (475 z units), in an immediately following observation, caused contraction. The rise in blood-pressure corresponding to the inhibitory reaction, however, was 22 mm. of Hg higher than that for the motor effect.

In another experiment, stimulation with a frequency of 5 per second (370 z units) resulted in a brief initial dilatation followed by well-marked contraction. The latent period for the occurrence of the relaxation was about 3 or 4 seconds, and the maximum extent of the succeeding volume decrease in the balloon was about 10 c.c. Increasing the frequency from 5 to 10 and from 5 to 15 per second, in this period of stimulation, resulted in each case in a diminution in the extent of the contraction, the partial relaxation being less for the former change than for the latter. In an immediately succeeding period of stimulation at 40 per second (370 z units), the initial quick relaxation took place, but it was followed by only a very slight and transient contraction. The latter fell far short of reaching the tonus level preceding stimulation. A succeeding relaxation of the stomach then occurred, becoming progressively greater till cessation of stimulation. The relaxation thus finally produced was considerably greater than that resulting from the initial dilatation. On cessation of stimulation, slow recovery took place, the tonus gradually rising to a level almost the same as that preceding stimulation. A brief initial dilatation followed by a temporary motor effect was observed also on crushing the celiac ganglion with forceps, and in one experiment, a similar reaction occurred on stimulating the fibres peripheral to the ganglion after removal of both adrenal glands.

In a, relatively, few instances, the more or less predominantly inhibitory reaction evoked by excitation of these fibres was followed by temporary contraction well above the level preceding stimulation. At times this motor after-effect occurred very abruptly and almost immediately after cessation of faradisation.

*Effects of stimulation of the splanchnic major in relation to vascular changes.*

The only definite effect of stimulation of the splanchnics on the lower end of the œsophagus, with adrenals removed or intact, was motor. The reaction was similar to that described for the fibres peripheral to the celiac ganglion, and it is illustrated in Fig. 3. The prolongation of the effect, after cessation of stimulation, was often longer than that there shown. In the case of the stomach, however, both motor and inhibitory reactions occurred. The motor effect was obtained in 13 of the 38 experiments, in animals to which no chemicals other than the anæsthetics



and Parke-Davis' adrenalin had been administered. In two of these, in which especially strong contraction took place, however, some concentrated sodium carbonate solution, the anticoagulant, had passed into the circulation. The result of splanchnic stimulation in one of the latter experiments is shown in Fig. 2. Whether one or the other effect takes place does not depend primarily on the frequency and intensity of faradisation, but on the extent of the change in the calibre of the blood vessels in the stomach wall, and to some extent, on the condition of the animal.

In experiments on 12 cats, with both vagi and both splanchnics cut, and with both adrenals removed, relaxation occurred in a total of about 50 observations, but contraction took place in 6 of the animals in a total of 25 observations. Blood-pressure was taken in 11 of these experiments, the anticoagulant being 4 p.c. sodium citrate. The former effect was produced by frequencies ranging from 1.8 to 225 per second and intensities varying from strong to weak to the tongue, *i.e.*, with secondary positions varying from 0 to 6 cm. with the Cambridge coil (in one observation, the intensity was 370 z units). The motor reaction was produced by very much the same frequencies and intensities, the former ranging from 2.5 to 185 per second, and the latter from strong to weak to the tongue (secondary positions from 0 to 5 cm.). Wider ranges of frequency and intensity were not tried for the two effects, except that in a few instances it was observed that a position of 6 cm. was about threshold. No definite combination of frequency and intensity could be predicted to give either effect.

In 45 of the observations in which relaxation occurred, simultaneous records of the blood-pressure were taken, and it was observed that the extent of the inhibitory reaction, in successive observations, closely paralleled that of the rise in blood-pressure, as illustrated in Fig. 1. Without a rise in blood-pressure, inhibition did not occur. At times, during rather prolonged stimulation, the tonus of the stomach began to rise, but concurrently the blood-pressure fell. In the experiment from which the figure was taken, the superior mesenteric artery and the abdominal aorta were tied. In all other experiments in which the splanchnic was stimulated, these vessels were not tied, but the results were in accord with those shown in Fig. 1.

The following series of observations may be mentioned in this connection, the intensity of stimulation remaining constant throughout (secondary at 2 cm.), and the animal being prepared with both vagi and both splanchnics cut, and with both adrenals removed. Stimulation of

the left splanchnic, with a frequency of 5.5 per second, caused a rise in blood-pressure of about 25 mm. and a fairly well maintained contraction

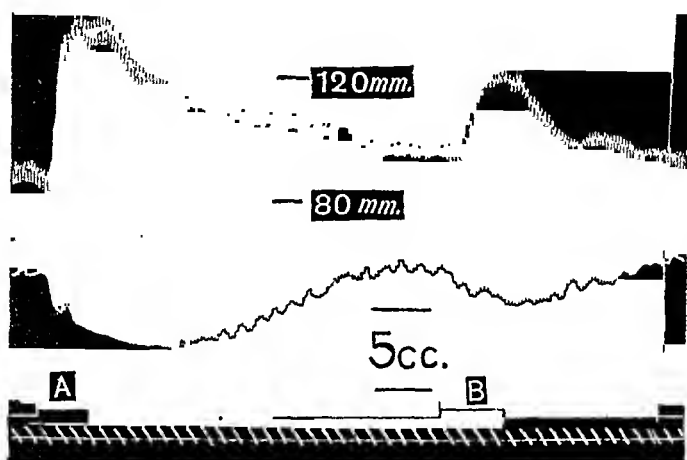


Fig. 1. Cat. Chloralose. Splanchnic majors and vagi cut. Adrenals removed. Superior mesenteric artery tied about 3 cm. distal to origin. Aorta tied between origin of renals and superior mesenteric. Viscera not exposed. Stimulation of right splanchnic. Shocks moderate to tongue (primary current = 0.09 amp.; secondary at 3 cm.). Upper tracing, carotid blood-pressure; lower tracing, stomach. A: 80 interruptions per sec. B: 5.5 per sec. Time in 10 sec. intervals. In this and following figures the distance between the short horizontal lines near the stomach tracing represents the indicated volume change in the stomach balloon.

of the stomach, resulting in an expulsion of about 3.5 c.c. of air from its balloon. Quickly increasing the frequency to 185 caused a further rise in blood-pressure of about 10 mm., and simultaneously the stomach relaxed, allowing an entrance of about 6 c.c. of air. In an immediately succeeding observation, 11.5 per second produced a fairly well maintained rise in blood-pressure of about 35 mm., but a diphasic response of the stomach occurred. It first contracted, expelling about 3.5 c.c. of air, but in the course of about 15 seconds, relaxation began to take place, finally permitting an ingress of about 6 c.c. In the third observation of this series, 3.3 per second evoked a rise in blood-pressure of about 22 mm., which was accompanied by a maintained 1 c.c. contraction of the stomach, persisting throughout the period of 95 seconds of stimulation.

Though these observations indicate that the effect produced on the stomach depends on the degree of vaso-constriction in its walls, they might also be considered analogous to Wedensky inhibition. The apparent analogy is offset, however, by the following result obtained in

another experiment, the animal being prepared in the same way. A frequency of 11.5 (secondary at 2 cm.) caused a rise in blood-pressure of about 45 mm., and this was accompanied by a back flow of 2 c.c. of air. The blood-pressure began to fall gradually in the course of about 20 seconds and simultaneously the stomach tonus rose to its preceding value. The frequency was then increased to 185 per second. The blood-pressure continued to fall until it reached a fairly constant level about 20 mm. above that preceding stimulation, and concurrently contraction of the stomach took place, resulting in an expulsion of 7.5 c.c. of air.

The most pronounced contraction in response to splanchnic stimulation occurred in an experiment in which both vagi and both splanchnic majors had been cut, and both adrenals removed, the record being taken soon after preparation of the animal. The anticoagulant was sodium citrate. In 40 seconds of faradisation of the left nerve, at 4 per second and with a secondary position of 3 cm., 33 c.c. of air were expelled from the stomach balloon and the intragastric pressure rose considerably more than 11 cm. of water. This increase could not be accurately measured by the method employed. The effect was purely motor from the outset, the contraction rising very smoothly, and a period of about 4 minutes was required for the stomach to return to its preceding tonus level. A motor effect of such great magnitude has been practically maximal, in the author's experiments, for excitatory stimulation of the vagus. The corresponding rise in blood-pressure amounted to about 18 mm., and it was quite well maintained throughout the period of stimulation. The effect on the lower end of the œsophagus was strongly motor, the tonic factor being predominant.

The nearest approximation to this contraction occurred on stimulating the splanchnic of an animal with both adrenals intact, both splanchnic majors and both vagi having been cut. A small amount of the anticoagulant, concentrated  $\text{Na}_2\text{CO}_3$  solution, had previously entered the circulation. A purely motor reaction developed, resulting in an expulsion of 27 c.c. from the balloon and an increase in intragastric pressure of considerably more than 9 cm. of water. The frequency in this observation was 160 per second, and the strength of the break shock was 370 z units. In this case the stimulation was without evident effect either on the blood-pressure or the lower end of the œsophagus. The reaction obtained is represented in Fig. 2.

In a number of experiments, the motor effect of the splanchnic became evident or well pronounced only under conditions in which it had lost, or almost lost, its ability to cause a rise in blood-pressure.

In one case, stimulation of the right splanchnic caused only relaxation of the stomach as long as a definite elevation in carotid pressure occurred.

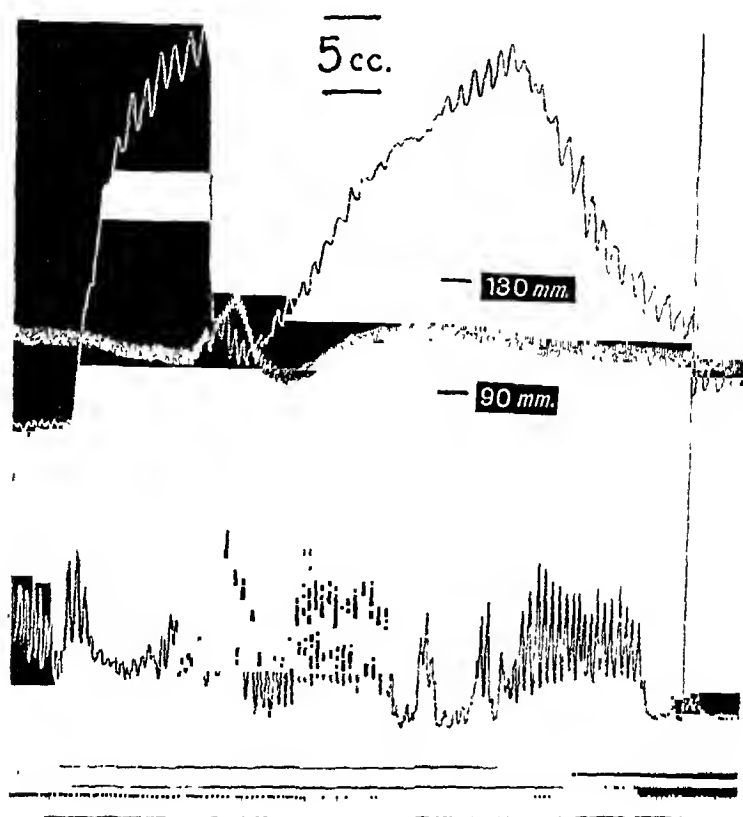


Fig. 2. Cat. Chloralose. Splanchnic majors and vagi cut; adrenals intact. Some concentrated sodium carbonate solution had previously entered the circulation. Upper tracing, stomach; middle, arterial blood-pressure; lower, lower end of oesophagus. Uppermost signal: stimulation of left splanchnic; 160 interruptions per sec.; strength of break shock, 370 z units. Lowest signal, injection of 2 c.c. of 1:100,000 Parke-Davis adrenalin (chloride) diluted with distilled water. Time in 5 sec. intervals.

As soon as this failed, however, the same intensity (secondary at 3 cm.) and frequency (2.5 per second) that had caused relaxation in a preceding observation, evoked well-marked contraction. In another experiment, contraction was produced by a frequency of 4.5 per second, the corresponding rise in blood-pressure being not more than 2 mm. Increasing the frequency to 23 per second resulted in a 4 mm. rise in blood-pressure and a pronounced diminution in the extent of the contraction. A similar

effect occurred, in the same period of stimulation, on a change from 4.5 to 72 per second, by throwing a switch in the primary circuit. The elevation in blood-pressure as a result of the frequency change was about 12 mm. The secondary position in this case was probably 3 cm.

In a preceding paragraph (p. 462) a diphasic response of the stomach to splanchnic stimulation was described. In this particular observation a transient contraction preceded relaxation, but such a reaction is very rare. It is more common to find that a more or less brief initial dilatation precedes contraction, but this type of response was observed in only 6 experiments, the vagi being cut in all. In each of 2 of these, the reaction occurred in only one observation. In the first, the left splanchnic major was cut and the left adrenal was removed, but the right splanchnic and adrenal were intact. Faradisation of the left splanchnic, with a frequency of 185 per second and a secondary position of 1.9 cm., produced an initial relaxation followed by contraction. A latent period of about 2 seconds preceded the dilatation. Its duration was about 5 seconds, and it resulted in an entrance of 2.5 c.c. of air into the balloon. Contraction then occurred, causing an expulsion of about 7 c.c. The stomach was not exposed to direct observation, and no record of blood-pressure was taken.

In the second experiment, both splanchnic majors had been cut, and both adrenals had been removed. The blood-pressure was very low and the heart beats were very weak, as indicated by the small extent of the corresponding oscillations of the mercury column. Under these conditions, stimulation of the left splanchnic resulted in a brief initial relaxation similar to that described in the preceding paragraph, the latent period being about five seconds. This was followed by contraction. The latter continued to increase for about a minute after cessation of stimulation, resulting in an additional output of air of about 6 c.c. The output during stimulation was about the same. This was the most pronounced motor after-action of which the author has either record or recollection from about 225 observations on stimulation of the splanchnic majors. As a rule, the beginning of relaxation from contraction coincides well with cessation of stimulation. The rise in carotid pressure, during the occurrence of the initial dilatation and the transition into contraction, was quite steady in this observation, and there was no indication of diminution in its extent for about 40 seconds of the total of 60 seconds of stimulation. It is improbable, however, that the carotid pressure, under such poor circulatory conditions, would have followed a quick

constriction of the stomach vessels corresponding to the brief initial relaxation.

In the other four experiments, this diphasic reaction was more constant in its occurrence, and the initial relaxation and the succeeding contraction, as a rule, were much more pronounced. The latent period for the occurrence of the relaxation in all observations was brief, usually amounting to about 5 seconds, as illustrated in Fig. 3. In one experiment,

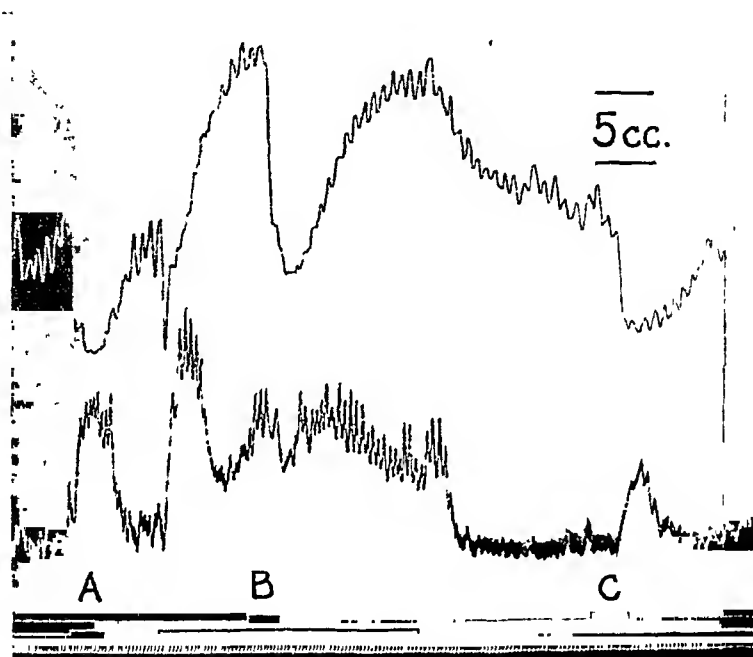


Fig. 3. Cat. Chloralose. Splanchnic majors and vagi cut; adrenals intact. Viscera exposed in moist chamber. Upper tracing: stomach. Lower tracing: lower end of oesophagus and cardia, balloon extending a few mm. below cardia. *A*: stimulation of right splanchnic; 40 interruptions per sec.; strength of break shocks, 145 z units. *B*: middle signal, stimulation of right splanchnic; 40 interruptions per sec.; strength of break shocks, 370 z units; upper signal, injection of 2 c.c. 1 : 100,000 Parke-Davis adrenalin diluted with distilled water. *C*: injection of 2 c.c. 1 : 100,000 Parke-Davis adrenalin. Time in 5 sec. intervals.

the response changed soon after removal of the adrenals into pure dilatation, but in another, their excision was followed by a change to pure contraction. In the latter, the blood-pressure was recorded, and the change, which consisted before removal of the glands of a brief rise followed by a slight fall, coincided well chronologically with the dilatation of the stomach. In the course of several seconds, however, the blood-

pressure returned to the level preceding stimulation, and about the same time, pronounced contraction developed. This condition persisted till the end of faradisation. After excision of the adrenals, the blood-pressure change was still limited to the beginning of stimulation, but it was greatly reduced, amounting to a rise of only 2 or 3 mm., and the effect on the stomach was motor from the outset.

In the remaining two experiments, a definite relation was found to exist between the effect produced and the intensity of stimulation. Blood-pressure was taken in neither, but in one of them the viscera were exposed to direct observation in the moist chamber described in a preceding paper<sup>(1)</sup>. The nerves in each case were stimulated with glass shielded electrodes. In the experiment in which the viscera were not exposed, both adrenals and the left splanchnic being intact, stimulation of the right splanchnic with a frequency of 40 per second and an intensity of 145 z units resulted in dilatation. This relaxation persisted during 40 seconds of stimulation, when a gradual regain of tonus began to take place. In the course of 50 seconds, the tonus had reached its preceding level despite the continued faradisation. Suddenly increasing the intensity to 370 z units, then, caused marked contraction without a preceding dilatation. The maximal extent of the dilatation in this period of faradisation was 5 c.c., and of the contraction 16 c.c. Later the left splanchnic was cut and stimulated, after removal of the right adrenal. Faradisation of this nerve with a frequency of 40 per second and an intensity of 145 z units likewise produced pronounced dilatation of the stomach, permitting an entrance of 15 c.c. of air into its balloon, and persisting throughout a period of stimulation 70 seconds long. Faradisation afterwards, with the same frequency, but with an intensity of 370 z units, resulted first in an initial relaxation, with a latent period of about 3 seconds. This dilatation permitted a back flow of 5 c.c. of air. It persisted only about 5 seconds, however, pronounced contraction beginning then to take place. The latter continued to develop till the end of stimulation, resulting in an expulsion of 27 c.c. of air, and an increase in intragastric pressure of considerably more than 9 cm. of water.

The results of stimulation of the left splanchnic were similar to those obtained in the experiment in which the stomach was watched directly. The tracings represented in Fig. 3, taken from this experiment, are typical of these reactions. Both splanchnic majors were cut, but both adrenals were intact, and the right splanchnic was stimulated in the thorax. An intensity of 265 z units and a frequency of 60 per second

had given repeatedly a reaction similar to that shown in Fig. 3, *B*, the injection of adrenalin during the motor response of the stomach having the same effect as that there shown. After taking the tracings given in the figure, moreover, observations *A* and *B* were repeated, with the exception of the adrenalin injection, with the same results. The position of the electrodes, in the course of the experiment, was not changed.

The vascular changes were of special interest when taken into consideration with the reactions of the stomach musculature. When persistent dilatation occurred, in response to 40 per second and 145 z units, the blood vessels constricted, resulting in pallor of the stomach wall, and peristaltic activity ceased. The bulging of the stomach was quite evident. The vaso-constriction persisted, furthermore, till cessation of stimulation, when the vessels began gradually to dilate to their preceding calibre. At the same time, contraction of the stomach and a return of peristalsis became evident. With the stronger stimulation, however, the vaso-constriction and the dilatation of the stomach were limited to the beginning of faradisation. This inhibitory effect was recovered from quickly, the entire body of the stomach entering into a gradually developing tonic constriction. Simultaneously the blood vessels dilated to the calibre preceding stimulation, and slowly moving peristaltic contractions appeared. With the further development of the motor effect, these peristaltic waves progressed more rapidly toward the pars pylorica. The contraction consisted chiefly of a definite circular constriction of the distended body of the stomach, gradual relaxation being evident, on cessation of stimulation, to the size preceding excitation of the nerve. Shortening in the longitudinal dimension was not ascertained, nor was it determined whether the motor reaction involved the pars pylorica. Peristalsis was evident in the pars pylorica, however, soon after relaxation of the blood vessels from the initial constriction, though during the vasoconstriction, rhythmic activity in this part of the stomach had ceased. Whether these different reactions might have been related also to frequency of stimulation was not determined.

These observations show conclusively that the contraction of the stomach by splanchnic action is not the result of mechanical compression produced by excitation of the diaphragm or other skeletal muscles or their nerves by spread of current. No observation, throughout the course of the investigation, was considered valid in which there was any evidence whatever of such spread. When the splanchnic is stimulated in the thorax, the diaphragm usually lies directly on the electrode shield. Direct observation of this part of the structure, through the incision in



the thoracic wall, shows that it remains quiescent while the stomach contracts (cp. (6)).

Since current spread is insufficient to excite the diaphragm or its intramuscular nerves in the conditions just mentioned, the presumption that the motor reaction might be the result of excitation of the vagus by such means is removed beyond the realm of probability. It is rendered still further unlikely when it is considered that the strongest contraction was obtained when the left splanchnic was stimulated beneath the diaphragm. The peripheral end of the glass electrode shield was at least 1 cm. from the unisolated coeliac ganglion. The shield was separated from the stomach by the cephalic pole of the left kidney, and usually under such conditions a layer of intact peritoneum adds to the completeness of the separation. Yet the contraction evoked compared favourably in magnitude with the strongest produced by stimulation of the vagus trunk, in the author's experiments, under the most favourable conditions. The shocks used, moreover, were only moderately strong to the tongue, and by no means painful. Stimulation of the right splanchnic with glass shielded electrodes, was observed to cause contraction with stimuli weak to the tongue, and in this case the possibility of effective current spread was further reduced by the intervention of the liver. In addition, stimulation of the splanchnic in the thorax may cause pronounced contraction of the stomach when the lower end of the œsophagus is unaffected. This state of affairs is illustrated fairly well in Fig 2. If vagus fibres to the stomach were excited by current spread, under these conditions, fibres to the lower end of the œsophagus would also be stimulated.

It may be mentioned, finally, that tying and exerting slight tension on the left splanchnic produced well marked contraction of the stomach in one case, the vagi having been cut. The most pronounced contraction in response to mechanical stimulation, however, was obtained in the experiment which gave the strongest contraction to electrical stimulation (see p. 463). About 15 minutes after this observation, sliding the shielded electrodes several mm. along the splanchnic, toward the coeliac ganglion, had a motor effect causing the expulsion of 23 c.c. of air.

### *Effects of adrenin.*

In about 10 observations in some 18 experiments, Parke-Davis' adrenalin chloride, diluted usually with distilled water, but infrequently with Ringer solution, was injected intravenously in doses varying from 1 c.c. of 1 : 100,000 to  $\frac{1}{2}$  c.c. of 1 : 1000. The result quite uniformly was relaxation of the stomach, often with complete cessation of peristalsis, and contraction of the lower end of the œsophagus, the extent of the reaction being proportional to the amount injected. In some cases, however, the latter structure was apparently not affected. From direct inspection by the moist chamber method (1), the impression was gained that the vaso-constriction produced in the stomach by the drug was not so great, with the smaller doses especially, as that which could be obtained by splanchnic stimulation. These reactions occurred whether the splanchnics were cut or intact, or whether the adrenals were removed or in place. Inhibition of the stomach occurred, furthermore, when it was contracted in response either to vagus or splanchnic stimulation.

had given repeatedly a reaction similar to that shown in Fig. 3, *B*, the injection of adrenalin during the motor response of the stomach having the same effect as that there shown. After taking the tracings given in the figure, moreover, observations *A* and *B* were repeated, with the exception of the adrenalin injection, with the same results. The position of the electrodes, in the course of the experiment, was not changed.

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contraction was maximal for a dose of 5 c.c. of 1 : 100,000, amounting then to an output of only 3 c.c. of air. The reaction to this dose is represented in Fig. 4. Larger doses only prolonged the reaction. The effect on the lower end of the œsophagus was, as usual, pronounced contraction. In the same experiment, the stomach was thrown into strong contraction by stimulation of the left vagus, and 1 c.c. of 1 : 100,000 was then injected. Marked relaxation took place, though the level reached was somewhat higher than that preceding stimulation. Recovery took place in about 30 seconds, and the stomach began to contract in response to the continued vagus stimulation.

In some observations, the adrenal glands being intact, pronounced relaxation of the stomach, in response to splanchnic stimulation, did not take place till the secondary rise in blood-pressure occurred. Anrep<sup>(7)</sup> has shown this secondary rise to be the result of an output of adrenin, and inasmuch as it coincided well with the accentuation of the inhibitory reaction on the stomach, the latter was considered to have, in part at least, the same cause.

*Parallelism between effects of splanchnic stimulation and anæmia  
on the stomach.*

The close relation of splanchnic effects on the stomach to the changes in the calibre of its blood vessels described above (p. 460 et seq.), suggested that the inhibitory reaction might be the result of anæmia. To test this probability, the aorta was occluded cephalad of the level of the apex of the heart, and the effects on the spontaneous activity of the stomach and the contraction produced by vagus stimulation were recorded. They were found decidedly similar to the action of the splanchnic under these conditions. Five experiments were performed. In four, the vagi and splanchnic majors were cut, and the adrenals were removed. In the fifth, the vagi only were cut, the splanchnics and adrenals remaining intact, and the results of this experiment will be considered separately. In all cases in which the vagus was stimulated, the left was chosen, and its cardiac branches were cut to prevent any diminution in its effectiveness by simultaneous inhibition of the heart. The following description is taken entirely from tracings. The stomach was not watched directly.

Occlusion of the aorta has much the same effect on the stomach when spontaneously active and when contracted during vagus stimulation. Relaxation takes place at once and peristalsis ceases. On release of the aorta, there is a more or less rapid return to the condition preceding the occlusion, as illustrated in Fig. 5. In another experiment in which the

reaction of the spontaneously active stomach was recorded, the regain of tonus was more rapid than that shown in Fig. 5, and there was a

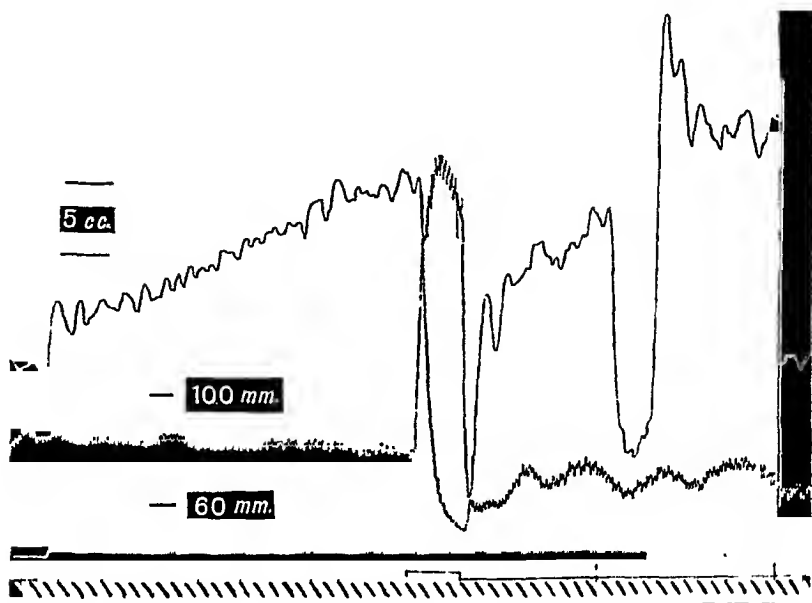


Fig. 5. Cat. Chloralose. Splanchnic majors, vagi, and cardiac branches of left vagus cut. Adrenals removed. Aorta clamped between origins of renal arteries. Viscera not exposed. Upper tracing, stomach; lower, carotid blood-pressure. Uppermost signal: stimulation of left vagus with shocks moderate to tongue; 1.03 interruptions per sec. to second upstroke in middle signal line, then quickly changed to 15.5 per sec. First signal on middle line: occlusion of thoracic aorta. Time in 10 sec. intervals.

tendency for the contraction to exceed temporarily the level preceding closure of the vessel. The longest period during which the blood flow was obstructed, in these two experiments, was about 60 seconds. In a third, however, in which the stomach was spontaneously quite inactive and atonic, closure of the aorta abolished the contraction in progress, in response to continued vagus stimulation, during a period of occlusion lasting 100 seconds. Longer closures were not tried.

Fig. 5 shows also the inhibitory effect of a relatively high frequency of vagus stimulation, in contrast to the excitatory effect of a lower frequency, without simultaneous inhibition of the heart (cp. (1)). No initial contraction preceded the relaxation. In a preceding observation, however, when the stomach was not contracted by excitation of the vagus, the same frequency and intensity of stimulation evoked a well-marked initial contraction, followed by inhibition. A strong motor

after-response followed the inhibition, similar to that represented in part in Fig. 5, and during this after-contraction, the aorta was occluded. The reaction was similar to that described in the preceding paragraph. The tonus on releasing the vessel, however, instead of rising only to the level preceding vagus stimulation, rose almost to the level of the after-contraction, *i.e.*, the motor after-response was resumed.

The inhibitory action of the splanchnic on the spontaneously active stomach has been considered in a preceding section and its inhibitory effect on the contraction produced by vagus stimulation is decidedly similar (Fig. 6). Concurrently with the rise in blood-pressure,

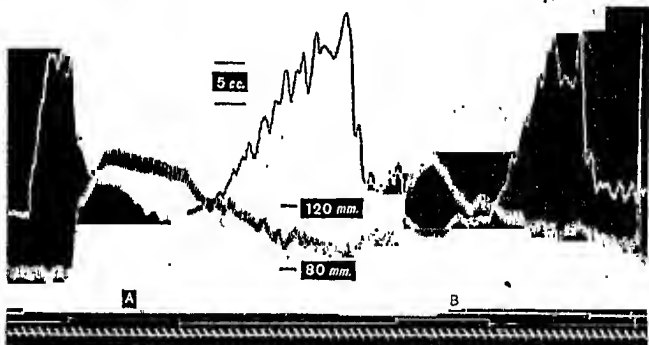


Fig. 6. Cat. Chloralose. Splanchnic majors, vagi, and cardiac branches of left vagus cut. Adrenals removed. Viscera not exposed. Upper tracing, stomach; lower, carotid blood-pressure. Uppermost signal line; stimulation of left vagus with shocks moderate to tongue;  $7\frac{1}{2}$  interruptions per sec. Middle signal line: stimulation of left splanchnic with shocks moderately strong to tongue (secondary at 3 cm., primary current .09 amp.); 60 interruptions per sec. Time in 10 sec. intervals.

the stomach relaxes, and if the spontaneous tonus before stimulation is pronounced, the relaxation proceeds to a level much below that preceding excitation of the vagus. On cessation of stimulation of the splanchnic, the rapidity of the development of the motor effect, in response to the continued stimulation of the vagus, corresponds well with the rate of fall in blood-pressure. This generalisation is made on the basis of five observations from two experiments. In an additional observation, the splanchnic was stimulated first and then the vagus. It is represented in Fig. 6, B. As long as splanchnic stimulation continued, the vagus

produced only a slight contraction. With the fall in blood-pressure, on cessation of splanchnic stimulation, however, the contraction developed more rapidly than in the corresponding part of observation A. Accordingly the fall in blood-pressure in the former case is extended over a shorter period of time.

In the experiment in which the splanchnics and adrenals were left intact, the effect of occlusion of the aorta on the contraction produced by vagus stimulation, in a single observation, was very much the same as that shown in Fig. 5. In this experiment, furthermore, the procedure was reversed. The aorta was occluded, and after the obstruction had been maintained about 30 seconds the vagus was stimulated. Only a slight trace of contraction was produced, resulting in an output of less than 1 c.c. of air. Stimulation was continued for 25 seconds, and about 20 seconds after its cessation, the aorta was released. The stomach contracted to a constant level in the course of 30 seconds, somewhat lower than that preceding deprivation of blood supply.

In these two observations, furthermore, there was evidence that occlusion of the aorta suppressed contraction of the lower end of the œsophagus. In the first observation, escape from vagal inhibition had taken place before the vessel was closed, but this procedure apparently brought about relaxation. After release of the aorta, contraction did not occur till cessation of stimulation. Then a strong motor after-response took place immediately. In the second, closure of the aorta apparently shortened decidedly the duration of the initial contraction, and the motor after-response was delayed in its appearance for some twenty seconds after release of the vessel. In the other experiments, closure of the aorta had apparently no effect on the activity of the lower end of the œsophagus, but it is possible, in these cases, that its blood supply was not greatly reduced.

#### *Effect of sodium carbonate on the response to splanchnic stimulation.*

It was stated in the section on the relation of splanchnic effects to vascular changes that a small amount of concentrated  $\text{Na}_2\text{CO}_3$  solution, the anti-coagulant, had entered the circulation in two experiments, and that in these, stimulation of the splanchnic caused strong contraction of the stomach. In one of them, furthermore, there was no corresponding change in carotid pressure whatever (Fig. 2). It seemed probable, therefore, that  $\text{Na}_2\text{CO}_3$ , when injected into the circulation, might permit a reproduction of these effects. This probability has been put to test in only three experiments, but the results have been, for the

most part, positive. In all cases, the splanchnic majors and the vagi were cut.

In the most doubtful experiment, the adrenals were left intact, and the injection of 0.5 c.c. of 0.5 N.  $\text{Na}_2\text{CO}_3$  apparently resulted in a change from inhibition to contraction during the primary rise in blood-pressure evoked by splanchnic stimulation. It did not abolish either phase of the blood-pressure change, however, and with the occurrence of the secondary rise, the stomach relaxed. Further injections gave indefinite results.

The other two experiments fulfilled expectation. In the animal, in which the adrenals were intact, the blood-pressure change in response to splanchnic stimulation was apparently completely abolished by injections totalling 5.8 c.c. of 0.1 N.  $\text{Na}_2\text{CO}_3$ , and at the same time, the reaction of the stomach became purely motor. This condition, however, was reached through stages of transition, the motor effect on the stomach appearing before complete disappearance of the rise in blood-pressure. The change brought about in the third experiment, after bilateral adrenalectomy, was similar to that produced in the second. In this case, moreover, initial dilatation of the stomach followed by contraction was obtained at times, after the injection, and the corresponding blood-pressure rise was transient and fairly well limited to the beginning of stimulation.

#### DISCUSSION.

The parallelism between the extent of inhibition of the stomach by splanchnic stimulation and that of the corresponding constriction of its blood vessels indicates that the important factor in the production of the inhibitory reaction is vasoconstriction. The occurrence of a motor reaction of the stomach when the vasoconstriction is slight or entirely lacking, or when the vessels relax during stimulation, points to the same conclusion. It is supported further by the fact that anæmia affects the stomach in much the same way as splanchnic stimulation, both when the organ is spontaneously active and when it is thrown into contraction by stimulation of the vagus. The results of occlusion of the thoracic aorta on the stomach are in accord with those of Bastianelli(8) for the dog, and with those of most investigators who have studied the effect of anæmia on the intestine. Bastianelli, Mall(9), and Bayliss and Starling(10) have reviewed preceding literature.

It is quite certain that the inhibitory and motor effects of splanchnic stimulation on the stomach are not the result of differential excitation of inhibitory and excitatory nerve fibres. In 36 of the 38 experiments,

neither effect could be certainly produced by any combination of frequency or intensity. In two experiments, a definite relation between intensity and reaction did appear. The results in both were the same, however, and direct inspection in one of them showed that the inhibition was closely paralleled by vasoconstriction. The similar parallelism in the other experiments, in which evidence for the presence of specific inhibitory nerves could not be obtained, indicates that in these two cases also, inhibition was the result of vasoconstriction.

The occurrence of an initial vasoconstriction with relatively strong stimulation, whereas weaker shocks of the same frequency produced continued constriction, suggests that under the proper experimental conditions, a Wedensky effect may be obtained on the blood vessels innervated by the splanchnic. Whether a similar effect can be brought about by increasing the frequency of stimulation remains to be determined. The brevity of the latent period (cp. (7)) and the duration of the initial constriction, and of the accompanying dilatation of the stomach, indicates that it is not the result of an output of adrenin. The results obtained by Langley and Dickenson<sup>(11)</sup> on the vessels of the buccofacial region of the dog, by stimulation of the cervical sympathetic, are of interest in this connection. Their clear description of the results should be consulted. In regard to intensity of stimulation at least, the dilatation is similar in its mode of production to Wedensky inhibition.

A number of cases of lack of parallelism between the action of adrenalin and that of the thoracico-lumbar autonomic nerves have been considered by Langley<sup>(12)</sup> and the papers cited contain references to related literature. The antagonistic action of this drug to the motor effect of the splanchnic on the cat's stomach may be added to the list.

The apparent effect of sodium carbonate, viz. prevention of splanchnic action on the blood vessels with the result that pure contraction is produced, the author intends to investigate further.

The tendency of the lower end of the œsophagus and stomach to return to a constant level of tonus (cp. also (1)), after having been inhibited or thrown into contraction by nervous action, indicates that their tonus is dependent on a constant state of excitation. If it were the result of the engagement of the two parts of a catch mechanism<sup>(13,14)</sup>, these structures would be expected to remain indefinitely in the condition imposed upon them by the extrinsic nerves.



## SUMMARY AND CONCLUSIONS.

1. The extent of inhibition of the cat's stomach by stimulation of the splanchnic major nerve parallels the degree of vasoconstriction produced within its walls (Fig. 1). If the constriction of the vessels is slight, or entirely lacking, contraction of the stomach often occurs (Figs. 2 and 3).

2. The effect of splanchnic stimulation on the lower end of the œsophagus is uniformly motor, though frequently it is apparently lacking.

3. Variation in frequency and intensity of stimulation fails to reveal the presence of specific inhibitory fibres in the splanchnic major trunk, before or after removal of the adrenal glands.

4. Effects of stimulation of fibres peripheral to the celiac ganglion are described.

5. The action of adrenalin on a stomach, which exhibits a definite degree of tonus, is inhibitory. In one experiment, in which the organ was atonic, a weak motor effect was the apparent result (Fig. 4). Injection of the drug also usually causes contraction of the lower end of the œsophagus (Figs. 3 and 4), but often it is without effect. In one experiment, in which the tonus and rhythmic activity were pronounced, however, brief inhibition occurred.

6. When the stomach is contracted in response to stimulation of the splanchnic major (Figs. 2 and 3), or of the vagus, adrenalin injection causes pronounced relaxation. An antagonistic action of the drug to the motor effect of these two nerves is thus demonstrated.

7. The inhibitory action of the splanchnic on the stomach, either when spontaneously active or when contracted during vagus stimulation (Fig. 6), is similar to that of anæmia produced by occlusion of the thoracic aorta under these conditions (Fig. 5).

8. A few observations indicate that the injection of sodium carbonate into the blood stream results in a disappearance of vasoconstriction in response to stimulation of the splanchnic major, and that simultaneously the effect of the nerve on the stomach becomes purely motor (cp. Fig. 2).

9. On the basis of this evidence, it is suggested that the chief factor in the production of inhibition of the cat's stomach, by stimulation of the splanchnic major nerve, is vasoconstriction.

The author wishes to express his gratitude for the grant of a Moscley Travelling Fellowship by the Harvard Medical School, and for the

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PROCEEDINGS  
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*March 21, 1925.*

**Modification of the Hewer method for staining in bulk with  
hæmatoxylin and eosin. By C. DA FANO.**

The bichromate solutions and the mixture of Muller's fluid, formalin and acetic acid proposed by Hewer<sup>(1)</sup> for fixing tissues were tried but did not appear to be entirely satisfactory for the purpose in view. After some tentative experiments it was found that a variety of objects could very well be stained in bulk with hæmatoxylin and eosin in about the same way as described by Hewer if they were fixed in Bouin's mixture of 75 c.cm. of saturated watery solution of picric acid, 25 c.cm. of formalin and 5 c.cm. of glacial acetic acid. Instead of acetic acid a saturated aqueous solution of citric acid can be used.

The *modus operandi* is as follows. Fix relatively small pieces of fresh tissues in Bouin's fluid for about 24 hours. Transfer them into 70 or 80 p.c. alcohol overnight and then into fresh alcohol of the same strength to which 1 p.c. of lithium iodide has been added. This mixture should be renewed every day until the picric acid is practically entirely removed. Wash the pieces in running tap water overnight and for some hours in distilled water. Transfer them into Scott's hæmatoxylin<sup>(2)</sup> diluted with three parts of either distilled water or 2 p.e. of acetic acid as suggested by Hewer. The pieces remain in the dilute hæmatoxylin from a fortnight to three weeks according to their thickness, more or less compact structure and state of development, while the hæmatoxylin is changed once at the end of the first week. After a quick wash the material is kept for two or three hours in 0.5-1 p.e. HCl in 70 p.c. alcohol and washed in tap water overnight. It is then passed through the ascending series of alcohols up to 95 p.e. alcohol. This is finally replaced with a 1 p.c. solution of eosin in alcohol of the same strength and the pieces are left therein for about a week. They are then washed for some hours up to 24 in 95 p.c. alcohol, dehydrated in absolute alcohol, cleared in xylol and embedded in paraffin. The sections can be stuck to slides by

the albumin method or mounted one by one by simply dissolving the paraffin with xylol on the slide.

The method has, so far, been successfully employed for various tissues and organs of man and mammals generally used for class purposes as well as for embryonic material and tissues of lower vertebrates and some invertebrates. It is likewise recommended for the brain from relatively small animals such as rabbits and rats particularly when many serial sections must be rapidly examined, as occurs, for instance, during investigations on the experimental transmission of the virus of herpes or of that of epidemic encephalitis. In this case however the following precautions are necessary: (1) the brains are fixed *in toto* and after some hours subdivided into slices 5–10 mm. thick in order that fixation should be prompt and uniform; (2) the slices are left in the fixing fluid for about two days and in the diluted Scott's hæmatoxylin for no less than a month; (3) for the counterstaining a 1 p.c. watery solution of eosin is used before passing the pieces through the ascending series of alcohols and embedding them in paraffin as usual.

(1) Hewer, E. E. Journ. Roy. Micr. Soc. p. 180. 1920.

(2) Scott, S. G. Journ. Path. and Bact. 16, p. 390. 1911–12.

### **The hexose-phosphate metabolism in skeletal muscle in hyperglycæmia.** By T. H. MILROY (*Preliminary communication*).

The importance of the synthetic processes in carbohydrate metabolism in skeletal and cardiac muscle requires to be borne in mind in the study of hyperglycæmias of different origin. The effects produced by various types of hyperglycæmia on the hexose-phosphate (lactacidogen) metabolism in rabbit's muscle have been investigated. The amount of phosphoric acid in the form of the ester was determined by Embden's method from the preformed and the sum of the preformed and the hydrolysed hexose-phosphate in the same muscles of the normal and hyperglycæmic animal. Practically always the amount of the hexose-phosphate is smaller in hyperglycæmic than in normal muscles, and may only be  $\frac{1}{3}$  or  $\frac{1}{4}$  of the latter. A much more constant and characteristic difference is, however, to be observed after the action of  $n/10$  sodium fluoride on the frozen and minced muscle in these cases. As has been shown by Embden and Lehnartz(1), the fluoride anion produces a great diminution in the amount of free phosphate in freshly minced muscle or in the expressed juice, and this is due to a synthesis of the

hexose-phosphate. This action can be greatly intensified by the addition of glycogen, but not dextrose, so that the free phosphate may almost entirely disappear. This action is greatly diminished in muscle obtained from animals rendered hyperglycæmic by adrenalin, ether anaesthesia, or cooling the pancreas. The following distribution of the phosphate in freshly minced frozen pale muscles of the rabbit may be taken as an average, calculated for the moist substance: 0.25 p.c. free  $\text{H}_3\text{PO}_4$ , 0.60 p.c. free and combined  $\text{H}_3\text{PO}_4$  or 0.35 p.c. in the combined form.

When such muscle is subjected to the action of the fluoride solution for 1-3 hours at room temperature, the free  $\text{H}_3\text{PO}_4$  falls on an average to 0.05-0.08 p.c. When the same procedure is adopted with minced muscle from hyperglycæmic animals, the fluoride synthetic effect is greatly diminished. The original free  $\text{H}_3\text{PO}_4$  value may remain unaltered (no synthesis), or it may be brought down at most to  $\frac{1}{2}$  of its original value in place of  $\frac{1}{3}$  or  $\frac{1}{4}$ . In severe cases of hyperglycæmia not only may there be no synthesis, but the pre-existing phosphoric acid ester may break down in part or practically entirely. On adding 0.4 p.c. glycogen to the  $n/10$  fluoride, the synthesis can be increased but not to the same extent as in normal muscle.

The glycogen content of the hyperglycæmic muscles slowly decreases, and fluoride retards the rate of its disappearance, but there is no marked difference between normal and hyperglycæmic muscle in this case.

I have to thank A. E. Campbell and J. S. Loughridge for assistance in carrying out the analyses.

(1) Embden and Lehnartz. *Ztschr. f. physiol. Chem.* 134, p. 243. 1924.

**A comparison of the lactic acid contents of the mammalian heart and skeletal muscle after stimulation and in rigor mortis.** By L. N. KATZ and C. N. H. LONG (*Preliminary communication*).

Whilst the work of Fletcher and Hopkins<sup>(1)</sup>, Meyerhof<sup>(2)</sup> and A. V. Hill<sup>(3)</sup> has given us a very fair understanding of the lactic acid formation in skeletal muscle, both on stimulation and in rigor mortis, little or no information is available as to the part played by this substance in the metabolism of the heart.

Some unpublished experiments of Miss Arning on the lactic acid content of amphibian hearts and skeletal muscles in chloroform rigor showed that the concentration of lactic acid in the latter was about three times that in the former.

Since the whole question of lactic acid accumulation is bound up with the dependence, or otherwise, of the muscle on its contemporary oxygen supply, it was deemed advisable to investigate the problem more in detail from this point of view.

The first series of experiments was done on isolated muscles and hearts and on decapitated cats. In each case it was found that whereas the heart soon failed when the  $O_2$  supply was cut off the skeletal muscle continued to contract almost as well as when  $O_2$  was present. It would seem therefore that the heart shows a greater dependence on its contemporary  $O_2$  supply and does not readily go into "oxygen debt."

In the next series of experiments the lactic acid content of the heart and skeletal muscles was studied after both had been driven to exhaustion. It was found that the heart failed to respond with a synergic contraction when its lactic acid content was only about one-third that of a skeletal muscle driven to exhaustion. We conclude from this that a comparatively small accumulation of lactic acid will change the  $C_H$  of the heart muscle sufficiently to interfere with excitation and the conduction of the impulse. This view is borne out by some recent work of Andrus and Carter(4), Drury and Andrus(5), and Andrus(6) who have shown how susceptible the isolated heart is to a slight increase in hydrogen ion concentration. The skeletal muscle on the other hand is not so susceptible to such small increases in lactic acid content.

Finally, we have put both heart and skeletal muscle into rigor mortis either by incubation at  $37^\circ C$ . or by treatment with buffered caffeine solution. The results obtained indicate that under these conditions the heart only contains about half as much lactic acid as the muscle, even during the caffeine rigor when 0.7 p.c. lactic acid is found in the skeletal muscle.

Whether this inability to form as much lactic acid in rigor mortis is due to a lack of lactic acid precursor or whether the mechanism of its production is quite different in the heart and skeletal muscle, are points which we are at present investigating.

- (1) Fletcher and Hopkins. *Journ. Physiol.* 35, p. 247. 1907.
- (2) Meyerhof. *Erg. d. Physiol.* 22, p. 299. 1923.
- (3) A. V. Hill. *Phys. Reviews*, 2, p. 310. 1922; *Erg. d. Physiol.* 22, p. 299. 1923; *Science*, 60, p. 505. 1924.
- (4) Andrus and Carter. *Heart*, 11, p. 97. 1924.
- (5) Drury and Andrus. *Ibid.* 11, p. 389. 1924.
- (6) Andrus. *Journ. Physiol.* 59, p. 361. 1924.

**Animal quinoidine.** By H. E. KINNERSLEY, R. A. PETERS  
and B. T. SQUIRES.

The blue fluorescence of the lens of the eye in ultraviolet light was described originally by Setchenow<sup>(1)</sup>, a pupil of Helmholtz, and that of urine by Schliess and Lowenfeld<sup>(2)</sup>. Bence Jones<sup>(3)</sup> showed that substances fluorescing like quinine could be extracted by animal tissues. We have examined a number of pure substances of physiological significance, as well as many animal and plant products for blue fluorescence in the ultraviolet light,  $\lambda$ .v. 3000–4000, using a screen of Chance's glass and a Hg lamp. No pure substance examined fluoresced blue, among which may be mentioned especially amino acids, purine derivatives, certain bile derivatives and cholesterol. The substance fluorescing blue in urine fractionates with the urochrome fraction of Garrod. A similar body can be extracted from guano, also from milk, liver, hen feathers. An acetone extract of grass also fluoresces blue after removal of chlorophyll.

The substances are not considered to be porphyrins. Linseed and cotton seed oil fluoresce more powerfully than olive oil. It is not clear whether the fluorescence is due to the same constituent in all cases. In the case of the animal there seem to be two types of substances, one which is either initially soluble in alcohol ether mixture or can be made so by treatment with potash, present especially in urine; the other found associated with skin, lens and gelatin does not respond in the same way to a similar treatment.

(1) Setchenow. *Archiv Opt.* 5. 205. 1859.

(2) Schliess and Lowenfeld. *Schmidt Jahr.* 120. 10. 1863.

(3) Bence Jones. *Chem. News.* 13. 197. 1866.

**Insulin and micro-organisms.** By L. B. WINTER and W. SMITH.

It has been shown<sup>(1)</sup> that an alcoholic extract of commercial yeast may cause a lowering of the blood sugar when injected into normal rabbits. When such an extract was injected into diabetic persons the action was similar to that produced by insulin<sup>(2)</sup>. In a series of further experiments with Dr H. B. Hutchinson different strains of yeast were separated from a commercial sample and grown in pure culture. Active and inactive cultures were obtained. By altering the conditions of nutrition it was not possible to obtain an active extract from a culture which was originally inactive. In the course of some months the active

strains completely lost the power of forming the insulin-like substance, although growth was vigorous.

Further work with the coli-form bacillus which formed an insulin-like substance(3) showed that this property was also gradually lost when the bacillus was grown in pure culture. The organism was still capable of rapid growth, but no lowering of the blood sugar took place when an extract was injected into rabbits. At this stage lactose was substituted for glucose in the culture medium. Activity was at once regained. When the organism was sown into solutions of peptone water containing glucose and lactose respectively, the lactose series gave extracts which sent normal rabbits into insulin-like convulsions (relieved by glucose); the glucose series had no effect on the blood sugar. After an interval of some months the power of forming an insulin-like substance was lost; this power was not regained when different sources of nitrogen and other sugars were used.

(1) Hutchinson, Smith and Winter. *Biochem. Journ.* 17. 683. 1923.

(2) Winter and Smith. *Brit. Med. Journ.* 1. 711. 1923.

(3) Hutchinson, Smith and Winter. *Biochem. Journ.* 17. 764. 1923.

### **The equilibrium of oxygen and hæmoglobin.** By G. S. ADAIR.

The remarkable S-shaped curve obtained, when the oxygen saturation of hæmoglobin is plotted against the oxygen pressure, is explained by Prof. A. V. Hill's theory(1), with the aid of the fundamental assumption that the osmotic pressure of hæmoglobin is  $1/n$ th of what it should be, if each hæmoglobin molecule contained one atom of iron.

Experimental evidence on this point was obtained by measuring the osmotic pressure and the oxygen curves of hæmoglobin solutions of different concentrations.

A fair test of the theory requires that corrections should be made for the volume effects of the protein, the so-called Donnan equilibrium, and the acid produced on oxidation. The lengthy investigations required will be described elsewhere. The corrected results are given below:

Oxygen capacities	29.3	21.9	15.0	7.5	7.3
$n$ osmotic	2.24	2.74	3.18	3.62	3.63
$n$ Hill's equation	2.50	2.0	2.16	1.72	1.80

Both methods agree in giving an  $n$  much larger than unity, but the quantitative differences are far greater than the experimental errors. Moreover, the arguments of Brown and Hill(1) are open to the type of objection urged by Prof. Hill(2) himself in his criticism of Loeb's work. The relation between the oxygen pressure and the temperature is governed by the second law of thermodynamics. Any theory which enabled us to



deduce the half saturation pressure  $x'$ , from observed pressures and saturations would enable us to calculate the heat of reaction, by the formula

$$\partial \log x' / \partial T = Q/RT^2.$$

[Hill uses  $-\partial \log K / \partial T = q/RT^2$ .]

As the  $K$  of Hill's equation,  $y = Kx^n/(1 + Kx^n)$ , is given by the relation  $n \log x' = -\log K$ , it is not surprising that their calculated heat should be  $n$  times as large as the observed heat. These points reopen the question of the molecular weight of pure hæmoglobin, which was considered as settled many years ago (3), because the arguments based on oxygen curves and heats of reaction cannot be admitted as proofs of the figure 16,700. The true value is probably four times as large.

(1) Brown and Hill. Proc. Roy. Soc. B. 94. 297. 1922.

(2) Hill. Ibid. A. 102. 705. 1923.

(3) Barcroft and Hill. This Journ. 39. 411. 1910.

### On the physiology of the so-called psycho-galvanic reflex.

By F. AVELING, R. J. S. McDOWALL and H. M. WELLS.

In a chloralosed cat changes in the resistance of the skin can readily be produced by stimuli which would bring about a similar fall of skin resistance in man. Such procedures usually cause a rise in arterial pressure and, as shown by one of us (R. J. S. McD.), a dilatation of the pupil. A fall of skin resistance can, in the cat, be produced by drugs such as adrenaline which constrict the skin vessels, by cold, or by hæmorrhage, while a rise is caused by drugs, such as acetyl-choline and amyl-nitrite or by mechanical procedures which dilate the peripheral vessels, such as the raising of venous pressure. Atropine does not abolish the changes unless large doses are administered. This and other evidence, *e.g.* the effect of cold, confirms the conclusions of Waller that the reflex is not due to sweat secretion.

It is concluded that the diminished skin resistance is due to constriction of the skin vessels in response to various stimuli and the fact that the reflexes most readily elicited from the palms is to be explained by the fact that the superficial capillaries in this region are less liable to be affected by cold and pressure of the electrodes.

The fall in resistance due to sensory stimuli can be brought about in the decerebrate animal which indicates that the higher mechanisms are not essential, and it is to be presumed that the reflex is due to one

of the primitive vasomotor reactions associated with protection of the animal and anticipated activity. Such a mechanism may be effected by the higher centres, just as we see in the case of the circulation and respiration. The term "psycho-galvanic reflex" is, then, too limited to be strictly accurate and "skin constrictor reflex" should be substituted.

**The influence of the sympathetic, parasympathetic and somatic systems of nerves on the tonus of muscle in the intact and decerebrate cat.** By A. ST G. HUGGETT and J. MELLANBY.

In a previous communication we gave an account of experimental results which indicated that adrenalin has no action on reflexes other than a specific effect on the respiratory centre resulting in a transitory condition of partial or complete apnoea. We found no evidence in support of Hunter and Royle's hypothesis that the plastic tonus of voluntary muscle is controlled by the sympathetic nervous system. In view of the importance of Hunter and Royle's views we have extended our observations to determine the effect not only of the sympathetic but also of the parasympathetic and somatic systems of nerves on the normal tonus of voluntary muscle and the exaggerated postural tonus of the decerebrate cat. In these investigations we have made use of the broad generalisations that in physiological doses (a) adrenalin and ergotamine stimulate and paralyse respectively the motor nerve endings of the sympathetic system, (b) pilocarpine and atropine stimulate and paralyse respectively the motor nerve endings of the parasympathetic system, and (c) curare paralyses the motor nerve endings of the somatic system of nerves. Plastic tonus in the decerebrate animals was estimated from the lengthening and shortening reactions. In the majority of the experiments the time relations of the knee jerk were directly recorded.

*Sympathetic system.* The intravenous injection of adrenalin (1 mgm.) does not influence the time relations or the strength of contraction of the quadriceps extensor muscle whether evoked directly by stimulation of the anterior crural nerve or reflexly by stimulation of the patellar tendon. We have never observed after the injection of adrenalin the production of an exaggerated tonus such as is found in the decerebrate cat nor indeed any increase in the tonus of muscle. In confirmation of these results an intravenous injection of ergotamine (.5 mgm.), a quantity which paralyses the motor side of the sympathetic system, produces no change in the rigidity of the muscles of a decerebrate cat nor in the time relations of the contraction of the quadriceps extensor pro-

duced reflexly by stimulation of the patellar tendon. A similar absence of effect is observed in the muscles of the intact anaesthetised cat. The intravenous injection of a lethal quantity of ergotamine (2.0 mgm.) annuls decerebrate rigidity but at the same time abolishes all reflex muscular contractions, and within 30 minutes the animal dies of respiratory failure in a manner similar to that observed in a decerebrate cat with its vagi cut. In fact, a lethal dose of ergotamine paralyses the reflex nervous mechanisms of the animal.

*Parasympathetic system.* An intravenous injection of atropine (5 mgm.) has no effect on the muscle tonus of the intact anaesthetised cat or on the rigidity of the muscles of the decerebrate cat. A similar absence of effect is observed after the intravenous injection of pilocarpine (2 mgm.).

*Somatic system.* The intravenous injection of curare (5 mgm.) into a decerebrate cat results in the immediate disappearance of all rigidity and the production of complete flaccidity of the muscles. Within two minutes, reflex response to a strong stimulus disappears and one minute later paralysis of the diaphragm ensues. This quantity of curare does not paralyse the sympathetic system of nerves since a typical adrenalin rise of blood pressure may be produced in a completely curarised decerebrate cat under artificial respiration.

On Hunter and Royle's hypothesis adrenalin and ergotamine should increase and diminish respectively, the plastic tonus of voluntary muscle. The absence of any demonstrable effect directly negatives their hypothesis that plastic tonus is due to impulses passing to the voluntary muscles through the sympathetic nervous system. Similarly the absence of effect of pilocarpine and atropine on muscle tonus indicates that the parasympathetics do not control the tonus of voluntary muscle. On the other hand, the immediate effect of curare in abolishing the rigidity of the muscles of a decerebrate cat when used in a quantity which has no effect on the sympathetic or parasympathetic system of nerves, indicates that this rigidity is due to impulses passing to the voluntary muscles through the somatic system of nerves. In fact the normal tonus of muscle and the exaggerated postural tonus of the muscles of the decerebrate cat are the immediate result of impulses passing to the voluntary muscle through the somatic system of nerves.

**Action of atropine on the gut in vitro.** By J. C. HOET.

Atropine has a variable action on the gut. In very small quantities (0.1 mg. for a cat or rabbit) it inhibits the strong contractions set up by muscarine, pilocarpine or arecoline both *in vitro* and *in vivo*. Stronger doses (1 in 100 mg.) have a stimulating effect best marked in the cat: and still larger doses (0.1 grm. and more) cause paralysis of the bowel.

Magnus in his work on the isolated gut paid no attention to concentrations less than 1 mg. in 100 c.c. His results showed stimulation with "small" and paralysis with larger doses: later workers in his laboratory (V. Lidth de Jeude, Lilienstrand, Leheux) found that very small doses (0.01 mg. in 100 c.c.) gave in 50 p.c. of the cases inhibition of the isolated rabbit's gut: the amplitude of the contractions became less and in a few cases the tone fell. They use starving animals.

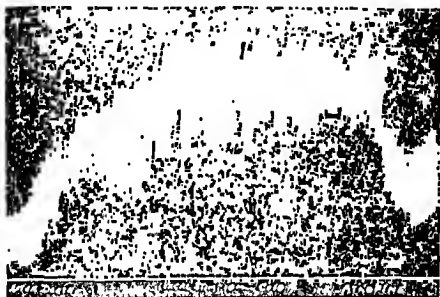
Lilienstrand found that there was no difference in the action of the optical varieties on the gut, and Lidth de Jeude that the explanation was not due to the composition of the saline medium or the concentrations of H ions. It was then thought that the variation might be due to changes in the composition of the gut. Leheux found from diffusion experiments that cholin was responsible for three-quarters of the intestinal activity, and concluded that the variation in the action of small doses of atropine depends on the choline content of the gut.

By keeping the gut on ice for 24 hours and washing with Ringer ten times or more he got a piece of gut which gave no reaction with 0.01 mg. of atropine. When he added 2 mg. of cholin to the bath an increase of movements occurred, which was inhibited by atropine.

Against this view the obvious objection occurs that intestines kept for 24 hours and washed undergo many other changes besides diffusion of cholin. I made three series of experiments to explain the effect of small doses of atropine. It is known that in the intact rabbit the inhibition of the movements of the gut produced by 1 mg. of atropine is more marked when the animal has previously had the vagi stimulated. In the first series of experiments both vagi were stimulated in a living rabbit for 10 minutes, after intravenous injection of 1 mg. atropine, the recognised view being that the vagal endings in the gut were not paralysed by such small doses. Isolated loops of this gut were allowed to record in Ringer and were found to be more regular and show less tone than the normal. The addition to the bath of 0.01 mg. atropine had no effect. No difference was noticed in the gut taken before and after vagus stimulation.

In a second series the animal was killed by bleeding: a normal piece of gut was first excised, the vagi were then stimulated from 6 to 10 minutes and then a second piece was excised. The difference between these pieces recording as isolated loops was compared. The tone of the stimulated gut increases in a few minutes, both the circular and longitudinal fibres showing great activity.

The unstimulated gut shows regular normal movements with no large variations and no increase of tone. Atropine  $1/5,000,000$  added to the bath gives a considerable fall of tone in the stimulated intestine, but is without effect on the normal loop.



In a third series of experiments, after excising a control loop, the vagi in a living rabbit were stimulated for alternative periods of 30 seconds. The difference between these loops is of the same nature as described above, although the tone of the stimulated loops was less marked than in the loops stimulated after death. Atropine  $1/5,000,000$  produces inhibition and a fall of tone in the stimulated loops but little or no effect in the control.

Dnodenal strips do not show these characteristic effects. In these experiments it is essential that the animal should not be killed in a state of asphyxia.

#### CONCLUSIONS.

1. Vagal stimulation in recently killed or living rabbits causes a change in the intestine shown in excised loops by increased tone of muscle and amplitude of contractions.

2. The action of atropine in very small doses on the intestine is determined by the state of innervation.

**The mercapturic acid synthesis in the dog.**

By H. I. COOMBS and T. S. HELE.

Since the time of Baumann it has been known that when a mono-halogen benzene is administered by mouth to a dog a rise of neutral sulphur occurs in the urine and the corresponding halogen phenylmercapturic acid can be isolated. Baumann was able to show that this mercapturic acid formation takes place in the para position to the halogen.

In a previous communication(1) it was stated that the administration of the ortho- and meta-dichlorobenzenes, compounds in which the para position is vacant, produces a similar rise of neutral sulphur. Unfortunately para-dichlorobenzene is not suitable for experiment owing to its toxicity but para-bromoanisole produces no rise of neutral sulphur. Is this due to the fact that the para position is occupied?

To test this point we administered the ortho-, meta- and para-chloroacetanilides and the corresponding chloroanisoles. In no case was there observed any rise of neutral sulphur though there was a large increase in the output of ethereal sulphate. It appears therefore, that the position of the substitutions on the benzene nucleus is not the only factor in the formation of a mercapturic acid. Whether this synthesis can only take place in a position para to a halogen is still unsettled.

The formation of para-chlorophenol as an intermediary between chlorobenzene and parachlorophenylmercapturic acid has always been assumed since the time of Baumann, but has never actually been proved. Experiments, which we have carried out, show this supposition to be incorrect. Para-chlorophenol given orally to a dog produced no rise of neutral sulphur in the urine.

The ortho- and meta-chlorophenols were equally ineffective, although the para position is unoccupied in these compounds. Phenol also produced no effect on the neutral sulphur.

Fluorobenzene, like chlorobenzene, brings about a rise of the neutral sulphur, but we have not as yet succeeded in isolating the corresponding mercapturic acid. Work is proceeding with iodothiophene, which has very similar physical and chemical properties to iodobenzene, and with other compounds.

(1) Hele and Callow. *Journ. Physiol.* 57; *Phys. Proc.* xliii. 1923.

PROCEEDINGS  
OF THE  
PHYSIOLOGICAL SOCIETY,  
*April 4, 1925.*

**On the numbering in series of sections from celloidin blocks.**

By C. DA FANO.

The following method is suggested for numbering and mounting in series sections from celloidin blocks. Pieces of any desirable size are embedded in celloidin by means of paper boxes or any similar device which may allow one to arrange them according to the way in which the series will have, afterwards, to progress. At the same time care should be taken that the pieces are surrounded by an amount of celloidin sufficient for subsequently writing a progressive number on the sections. The celloidin blocks are stuck to appropriate supports and these are fixed on the microtome as usual. A rather large and flat glass plate is prepared next to the microtome and covered with a sheet of thick blotting paper of the same size. This is thoroughly moistened with 70 p.c. alcohol. Numbered strips of thin smooth paper are then placed in a given order on the wet blotting paper and cutting is started. The sections are collected from the knife by means of a soft brush and placed in series on the paper strips. When a sufficient number of sections has been cut the first paper strip with its sections is lifted from the wet blotting paper and placed on a dry one. The sections are then gently pressed and dried a little with a piece of repeatedly folded filter paper. Without any loss of time a number is written on a corner of the celloidin surrounding the sections by means of a small good brush and a mixture of 10 c.cm. of Indian ink and 3 c.cm. of equal parts of anhydrous ether and acetone. The figures dry up instantaneously and become almost engraved in the celloidin. If the paper strip with the sections is moved about in a dish of 60 or 70 p.c. alcohol, the sections float in it. One then proceeds to number the sections placed on the second paper strip and so on until all sections are used. The numbers thus written on the celloidin are not deleted by water and common reagents such as alcohol, xylol, the Weigert mordant, the bath used for toning and fixing Golgi-Cox specimens

and so on. Many sections can, therefore, be stained and treated as desired at the same time and finally mounted according to the progression of their numbers provided, of course, that media which dissolve celloidin are not used. If it is imperative to dissolve the celloidin before definitely mounting the sections, the operation must be carried out on slides previously numbered by means of a diamond point in the same way as the sections.

**The purpose of tetany and convulsions.** (*Preliminary communication.*) By J. ARGYLL CAMPBELL.

Using a method of injection of gas under the skin and into the abdominal cavity, I(1) have shown that a few minutes' vigorous muscular exercise causes a marked rise (20-50 p.c.) of  $O_2$ -tension in the tissue spaces—that is in the fluid bathing the wall of the cells. This rise of  $O_2$ -tension is considered to be due mainly to the action of lactic acid upon the dissociation of  $HbO_2$  in the tissues.

I also found that following insulin convulsions there is a similar marked rise in  $O_2$ -tension in the tissues.

In further experiments I find that tremors and convulsions following thyro-parathyroidectomy<sup>1</sup> in cats and rabbits increase the  $O_2$ -tension in the tissue spaces by as much as 50 p.c.

From the above I conclude that tetany and convulsions indicate an attempt on the part of the organism to supply  $O_2$  at a higher tension for the cells; from this it may be deduced that the cause of tetany and convulsions is  $O_2$ -deficiency in the cells.  $O_2$ -deficiency in the cells may arise either because there is a decrease of  $O_2$ -tension in the fluid bathing the cell wall or because the cell—owing to poisoning, etc.—cannot “absorb”  $O_2$ .

Morris(2) has already introduced a theory that tetany is due to anoxæmia and proved that asphyxia, decrease of temperature, anæmia, alcohol and histamine cause an increase in the electrical excitability of the neuro-myone owing to anoxæmia. He did not establish this “anoxæmia” theory in the case of tetany following either guanidine poisoning or parathyroidectomy. In my experiments I have found that guanidine markedly lowers the  $O_2$ -tension in the tissue spaces before tetany is produced and as shown above, I have found that in tetany following parathyroidectomy the  $O_2$ -tension is markedly affected. Morris studied the changes in the oxygen content and “oxygen head” of the blood. By my method it is possible without anæsthesia to proceed a step nearer to the



cells and to examine the changes in  $O_2$ -tension in the fluids actually bathing the cell wall. In this way, I have studied so far the effects of excessive artificial respiration, parathyroidectomy, injection of  $NaHCO_3$ , of  $NaCl$  and of guanidine, which all cause marked changes in the  $O_2$ -tension in the tissues. The evidence produced indicates that tetany and convulsions are caused by  $O_2$ -deficiency in the cell and that the purpose of tetany and convulsions is to counteract this defect.

<sup>1</sup> Dr H. H. Dale and Dr J. H. Burn kindly performed the operations.

(1) Campbell. *This Journ.* 59. 395. 1925.

(2) Morris. *Brit. Journ. Exp. Path.* 3. 101. 1922.

# PROCEEDINGS

## OF THE

# PHYSIOLOGICAL SOCIETY,

### *May 23, 1925.*

**Insulin and sodium aceto-acetate.** By J. H. BURN.

The appearance of acetone bodies in the blood is usually supposed to be due to their abnormal formation when fat metabolism is deranged. Their presence has been recorded in diabetes, in starvation and in subjects on a carbohydrate-free diet.

Marks(1) has recently carried out experiments which suggest that the secretion of the pancreatic hormone is not continuous, but is dependent on a rise of blood sugar above the fasting value. Consequently in starvation, and in subjects on a carbohydrate-free diet, as well as in diabetes, there may be an absence of insulin from the circulating blood. It seemed possible that the occurrence of acetone bodies in these three conditions might be due, not to an abnormal formation, but to the accumulation of normal metabolic products which did not accumulate when insulin was present in the circulation.

I have carried out experiments in which sodium aceto-acetate, prepared by Hurtley's method(2), has been injected into the veins of decapitated cats, and have made observations on the effect of insulin on the rate of disappearance of the sodium aceto-acetate from the blood. The following are the details of such an experiment:

*Cat, 3.1 kgm.* Decapitation under ether anæsthesia, after which the animal was respired with air. Kidney vessels were tied on each side, and kidneys removed. Blood-pressure recorded from one carotid artery, and the other carotid prepared so that samples of blood could be drawn off. Injection of 30 c.c. of sodium aceto-acetate solution (in all 2.4 gm. pH 7.5) into ext. jug. vein. Samples of 2.0 c.c. blood were withdrawn at intervals and the total acetone bodies determined by the method of van Slyke and Fitz(3).

		Total acetone bodies
25 minutes after injection of Na aceto-acetate		54.5 mgms. per 100 c.c.
20    "    later		50.0           "
20    "    "		44.0           "
10 units insulin injected (i. v.)		
30 minutes later		37.0           "
30    "    "		32.0           "
30    "    "		25.0           "

Determination of the blood sugar, before and after the injection of insulin showed that the usual fall of blood sugar was produced by the injection

In the above experiment, as in three others, no effect of insulin on the rate of decline of the total acetone bodies in circulation was observed. The disappearance recorded was closely similar at all stages to the disappearance in control experiments in which no insulin was injected

- (1) Mair's Unpublished communication to the Physiological Society
- (2) Hurler Quart Journ Med 9 301 1916
- (3) Van Slyke and Fitz Journ Biol Chem 12 495 1917

### **Vascular reflexes, chiefly in the ear of the rabbit.**

(Preliminary communication) By J N LANGLEY

The fall of blood pressure in the rabbit caused by stimulating the depressor nerve is not in any part due to dilatation of the vessels of the ear, for the fall of pressure, though it is sometimes unaccompanied by any visible change in the ear, is usually accompanied by easily visible contraction of the arteries. The contraction is not wholly a passive effect, for it is greater in parts of the ear the sympathetic nerve fibres to which are intact than in those to which the sympathetic nerve fibres have been cut. Similarly the fall of blood pressure caused by stimulating the central end of the vagus in the cat is usually accompanied by pallor of the nostrils and lips and the pallor is greater on the side with intact sympathetic than in that on which the cervical sympathetic has been cut. The stimulation may also cause pallor of the penis and of the pads of the feet.

The results suggest that there are certain afferent fibres proceeding from the viscera which inhibit the part of the vaso constrictor centre connected with the viscera and excite the part connected with the skin. Whether the excitation is an indirect effect, set up peripherally or centrally by fall of blood pressure, remains to be determined. The fact that excision of the abdominal viscera does not essentially alter the results, is fairly good evidence that these afferent fibres cause dilatation of the arteries of the muscles. Dr Jarisch is investigating this question and so far he finds that in the rabbit the depressor causes increase of volume of the gastrocnemius muscle.

The vagus in the rabbit, as is known, commonly causes a rise of blood pressure. The effect on the ear varies with the depth of anaesthesia and the strength of the stimulus. At the beginning of an experiment there is

usually contraction of the ear vessels. Deep anæsthesia and a strong current favour dilatation—and this may be nearly maximal. The dilatation is largely due to a venous state of the blood, caused by cessation of respiration, but, though lessened, it occurs during artificial respiration. In this case also, it appears that the visceral and the cutaneous circulation may react in opposite ways.

The experiments were made on anæsthetised animals; the stimuli were at the rate of 35 to 40 interruptions a second of the primary current. Both vagi were cut.

### A note on the melanophore dilator action of the pituitary.

By H. H. KNAUS, N. B. DREYER and A. J. CLARK.

The writers compared the activity of a water soluble pituitary powder, kindly supplied by Messrs Parke Davis, with that of fresh gland extract made by the method of Burn and Dale. The water soluble powder was stated to have seven times the oxytocic activity of fresh gland, and this figure was confirmed on the isolated uterus of the guinea-pig. Experiments made on pithed cats, with the precautions described by Hogben, Schlapp and Macdonald(1) showed that 0.2 mgm. of the powder produced the same rise of blood-pressure as did 1.6 mgm. of fresh gland, that is, a ratio of 1 to 8.

The powder, however, had a much feebler relative action on the melanophores of the frog.

Dose of pituitary in mgm.  
per 25 grm. frog

		0.03	0.02	0.01	0.005	0.0025
Reactions produced by fresh gland	Black	2	1	—	—	—
	Very dark	—	2	—	—	—
	Dark	—	3	5	—	—
	No visible darkening	—	—	2	4	—
Reactions produced by dry powder	Black	3	3	3	—	—
	Very dark	—	—	2	3	—
	Dark	—	—	3	2	1
	No visible darkening	—	—	—	2	4

Observations were made on 44 frogs, and the responses were divided into four classes according to their intensity. The frogs were kept exposed to bright daylight, on a white background, in dry glass jars.

The results are shown in the table, and indicate that the dried pituitary powder has more than twice and less than three times as strong an action as fresh gland.

In another shorter series of experiments another preparation of fresh gland was found to have only one-tenth the oxytocic action of the dried powder, but to have at least half as much melanophore dilator action.

We consider that these results provide strong additional evidence for the view that the melanophore dilator principle is distinct from the oxytoxic and pressor principles.

(1) Hogbon, Schlapp and Macdonald. *Quart. Journ. of Exp. Phys.* 301. xiv. 1924.

The expenses of this research were defrayed in part by a grant from the Government Grant Committee of the Royal Society.

### The temperature coefficient of muscle viscosity.

By J. F. FULTON.

Attention has previously been directed to the fact<sup>1</sup> that when recorded with a myograph of high natural frequency the isometric twitch of intact skeletal muscle is characterised by a flat plateau which terminates abruptly (the "angle"). This sudden discontinuity together with the concave shape of the curve of relaxation suggests that the "angle" denotes the point of cessation of contractile activity and that the curve of relaxation represents the viscous return of the muscle to its resting shape. The effect of temperature on the rate of relaxation also favours this inference. In a large number of experiments in which the responses of the same preparation (intact gastrocnemius) have been taken at 10 and 20° the averaged  $Q_{10}$  of the interval between the beginning of the electrical response and the "angle" has been found to lie between 2.00 and 2.10, while the interval from the "angle" to half-relaxation has a  $Q_{10}$  between 1.3 and 1.4. Records from a typical experiment are plotted in Fig. 1.

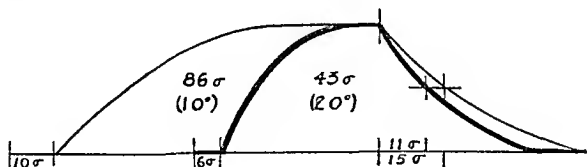


Fig. 1.

Two twitches from the intact gastrocnemius of a decerebrate frog at 10 and 20° are plotted on the same coordinates in such a way that their respective "angles" fall at the same point. The tension developed in the two was the same so that the only variable component of their

<sup>1</sup> *Proc. Roy. Soc.* 97 B, 424-431. 1925.

respective phases was duration. It will be seen that the duration of the interval between the electrical response and the "angle" falls from 86 to 43 $\sigma$  with a rise of 10°, while the duration of relaxation falls only from 15 to 11 $\sigma$ . The total interval between the action current and the beginning of shortening<sup>1</sup> (phases 3 and 4 of the latent period) is shortened from 10 to 6 $\sigma$  by a rise of 10°.

**Correlations between growth, differentiation and metabolic rate in the chicken embryo.** By HENRY A. MURRAY, Jr.

The present results were obtained during the course of a general investigation of physiological ontogeny, in which correlations were made during the embryological period of the chicken between changes in certain physiological phenomena, such as growth and metabolic rates, and in the chemical constitution of the tissues. The period under investigation extended from the 5th to the 19th day of incubation. The phenomena primarily examined were: (1) growth, (2) gross form development, (3) concentration of solids, and (4) chemical differentiation. It was found that there were significant phase differences, *i.e.* differences in the period when changes were rapid or the reverse. The rates of the first two functions (growth and form) changed rapidly in the beginning, whereas the latter (concentration and differentiation) changed mostly during the last half of incubation.

There were then two type rate curves which were almost opposite in form. They showed skew symmetry around a central point. This phase difference was the basis for distinguishing between primary, or gross, integration (growth) which occurred synchronously with primary, or gross, differentiation of form, and secondary, or internal, integration (concentration of solids) which was concomitant with internal differentiation of chemical form. These two groups are comparable to Spencer's division of the evolutionary process into primary and secondary redistributions. In this report it is being pointed out that they occur at rates which change at different periods in the life span.

A distinction on the basis of rate change differences was discovered between the extent of growth and the latent period of growth in culture experiments with tissues from embryos of different ages. Finally, it was found that the katabolic rate as judged by figures for CO<sub>2</sub> production was closely correlated with chemical composition. It was found to decrease as the percentage of water diminished, and the concentration of dry substance increased.

<sup>1</sup> *Journ. Physiol.* 59; *Proc. Physiol. Soc.* xlvj-xlviii. 1924.

**A special form of polarimeter tube. By L B WINTER**

Identification of phenyl osazones is much facilitated by determining the specific rotation and observing the mutarotation. When pyridine-alcohol mixture is used as the solvent, it is very difficult to make the latter determinations in an ordinary polarimeter tube with small quantities of material, the solution rapidly turns dark in colour by coming in contact with the metal cap, even the most accurately made tube seems to permit enough leakage for this darkening to take place, which was not prevented by fitting asbestos in place of rubber washers.

A glass tube approximately 1 dm in length was fitted at each end with a stopper into which a circle of polished glass had been cemented. A side tube with ordinary stopper is used for filling. It has been found that if the joints are carefully ground leakage is so slight that readings may be taken more than one day after the tube is first filled, and no darkening of the solution takes place. 3 cc are required to fill the tube. As it is difficult to determine the length by measurement, the tube is calibrated with a known sugar solution.

**A correlation of the size of the action current of skeletal muscle with length, tension and initial heat production.**

By J F FULTON

In a previous communication it was pointed out<sup>1</sup> that the size of the successive action currents of the intact gastrocnemius and sartorius muscles of the frog varied with the tension developed. Since then the relation between tension and length has been investigated further. In sartorius diminution in size of the action currents during a short tetanus can be prevented by placing the muscle under high initial stretch and arranging that the response shall be perfectly "isometric." In a completely "isometric" response of gastrocnemius, on the other hand, it is impossible to prevent successive diminution in size of the action-currents at any initial tension. This is interpreted as being due to the unpreventable shortening of the fibres in gastrocnemius owing to their diagonal disposition. Since the degree of diminution in size of the action-currents increases with increasing "isometric" shortening<sup>1</sup>, and since the tension developed diminishes correspondingly, it is clear that under these circumstances (but cf 1, p 414) the size of the action current as well as the tension developed diminish with the length of the fibre. If a muscle is forcibly lengthened during a tetanus the successive action currents

have been shown to increase in magnitude. Moreover, at maximal shortening of the completely unloaded muscle the size of the successive electrical responses during a tetanus is 50 to 60 p.c. less than when recording an "isometric" response at approximately its resting length.

It is evident from this that the size of the electrical responses vary qualitatively, and possibly even quantitatively with the initial heat production of muscle at various lengths as recently described by A. V. Hill. This clearly favours the view of contraction put forward by Hartree and Hill<sup>2</sup> (p. 140) in which they assume that a stimulus causes a momentary rise in permeability which is "manifested by the electric change," and, as already pointed out<sup>1</sup>, it suggests that the size of the action-current is a measure of the initial process of breakdown.

<sup>1</sup> *Proc. Roy. Soc.* 97 B, 406-423. 1925.

<sup>2</sup> *Journ. Physiol.* 55. 133-158. 1921.



6

PROCEEDINGS  
OF THE  
PHYSIOLOGICAL SOCIETY,  
*May 30, 1925.*

**The effects of insulin upon sugar utilisation in muscle.**

By C. G. LAMBIE.

Cats were used for the experiments. The general technique resembled that described by Dale and Burn<sup>(1)</sup>. The animals were decerebrated and eviscerated and the liver and kidneys tied off. The rate of sugar utilisation was studied by finding the rate at which it was necessary to inject glucose in order to compensate for the fall in blood sugar. The apparatus used for injecting glucose at a uniform rate consisted of a square screw held in a bracket and threaded through a small wheel driven by a motor. As the screw advanced slowly it pushed forward the piston of a record syringe. The apparatus could be reversed so that a second injection could be performed through a similar syringe at the other end, without much interference with the rate of flow. The speed was regulated so that 5 c.c. of fluid were delivered per hour, the requisite strength of glucose solution to give so many grammes per hour being placed in the syringe, which was connected by a fine rubber tube with a cannula in the jugular vein. This apparatus has the advantage that any resistance produced by kinks in tubing or veins is easily overcome so that the rate of injection is not appreciably interfered with.

The approximate rate of injection necessary to balance the fall in blood sugar averaged 0.15 gm. per kilo per hour. After 10 units of insulin per kilo the rate of glucose transfusion had to be increased by 75 p.c. to 100 p.c. to maintain a uniform level, the volume of fluid injected before and after insulin being kept constant.

The fall in blood sugar whether with or without insulin did not appear to be influenced by anaesthetics nor by pituitrin. A greater rate of injection was necessary to maintain a uniform level if the portal circulation were maintained intact and both splanchnic nerves cut. This may have been due to a better condition of the general circulation.

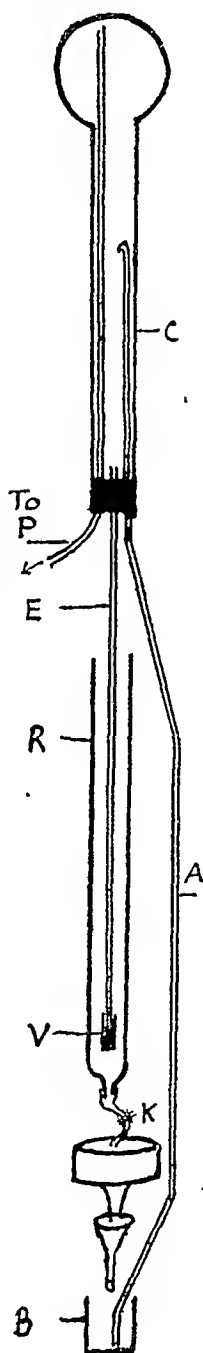
(1) Journ. Physiol. 59, 164. 1924.

**An automatic perfusion apparatus.** By W. O. KERMACK  
and C. G. LAMBIE.

The perfusion fluid is placed in the reservoir (*R*) from whence it passes by gravity through a rubber tube controlled by a screw clip (*K*) to the organ (*e.g.* liver) which is placed on a Büchner funnel. It collects below in a beaker or conical vessel and is then drawn up the tube (*A*) into the top reservoir (*C*) which is exhausted by means of a water pump (*P*). As the fluid is passing up *A*, along with air, it becomes automatically aerated. The froth which is formed rises up in *C* but breaks when it reaches the bulb at the top.

After the perfusion fluid rises to a certain height in *C* it gravitates down through *E* into *R* thus completing a cycle. The lower end of *E* is furnished with a modified Bunsen valve which prevents regurgitation. This valve consists of a piece of rubber tubing with a longitudinal slit like the ordinary type of Bunsen valve, but in addition it is covered with another piece of rather thicker tubing with a slit of approximately the same size. Provided that the rate of perfusion is not too rapid, the fluid in the reservoir *R* remains at about the same height while the rate of flow and the pressure are maintained at an approximately constant level. The rate is regulated by means of the screw clip *K*. The motive force is supplied by the water pump and by gravity. The rate of outflow may be measured by inserting a Condon's tipper below the glass funnel beneath the Büchner. Should it be desired to perfuse organs in series, another similar apparatus is set up alongside the one just described and, after separate perfusion, the tubes (*A*) are crossed to the other corresponding beakers (*B*). The fluid then circulates in figure of eight fashion. When limbs are to be perfused a large glass filter funnel, fixed at a convenient angle, is used instead of the Büchner.

The whole apparatus measures about 8 feet in height and is placed inside a hot chamber kept at body temperature. This gives freedom to carry out



any manipulation that may be necessary without interfering with the temperature.

The apparatus has been used to study the effects of insulin upon sugar utilisation in liver and limbs when these are perfused separately and in series. The fall in blood sugar was increased when insulin was added to the perfusion fluid passing through limbs alone, but no evidence could be obtained that this was any more marked if the blood had been previously passed through the liver with insulin. No effect could be demonstrated upon the rate of sugar production in the liver. The results of limb perfusion agreed with those described in another paper.

### **The action of the intercostal muscles. (*Preliminary note.*)**

By E. SHARPEY SCHAFFER and A. D. MACDONALD.

In an anæsthetised (or decerebrated) animal (dog, cat, rabbit) with respiration maintained by positive ventilation a complete hoop—including two adjacent ribs and rib cartilages, the intervening *museuli* intercostales and intercartilaginei and the segment of the sternum to which the two ribs are attached—is isolated from the rest of the thorax, except at the attachment of the ribs to the vertebral column, by cutting through all muscular attachments above and below the hoop, the isolation being completed by severing the *museles* right down to the spine. The nervous and vascular supply of the *museles* of the hoop are not interfered with and due precautions are taken to maintain the temperature both of the animal's body and of the isolated thoracic hoop.

If now the artificial respiration is diminished the natural movements of respiration are resumed and the isolated hoop moves forward in inspiration with those ribs above and below it which were left *in situ* and also with the descent of the diaphragm, and backward in expiration with the backward movement of the ribs left *in situ* and with the ascent of the diaphragm (contraction of abdominal *museles*).

On examining the preparation the *museuli* intercartilaginei can be distinctly seen and felt to be contracting with inspiration and relaxing with expiration. But it requires closer observation to detect contraction of the external intercostals, since they appear not to contract as a whole, except perhaps in extreme dyspnoea. And if the fibres of the muscular sheet are only contracting here and there it is difficult to detect their movement amongst the many resting fibres. Nevertheless it is sometimes distinct. And that they are always able by themselves without the assistance of the intercartilaginei to bring forward (raise) the ribs is shown

by the fact that this movement still occurs after the intercartilages are cut through. In this case the movement must be wholly due to the action of the external intercostals. For the internal intercostals cannot—owing to the direction of their fibres and the fact that they are put on the stretch when the ribs are moved forward—aid in this movement. On the contrary, they oppose it by their elasticity. Whether they take an active part in the backward or rib-depressing movement is difficult to determine. We have been unable to see or record such active contraction, but unless it were strong and involved all the fibres of any part of the muscular sheet, it would be difficult to detect, or to differentiate from the passive retraction which results from the fibres having been put on the stretch during the forward movement of the ribs.

We had supposed it would be easily possible to resolve this question of the active participation of the internal intercostals in the expiratory movement of the ribs by the employment of the string galvanometer, but have met with unexpected difficulties in applying that method, and have not yet succeeded in entirely overcoming these difficulties.

**A new colorimetric method for the estimation of glucose in blood.** (*Preliminary communication.*) By J. A. MILROY.

This method is based on the fact that any nitro-anthraquinone sulphonate when heated with glucose in alkaline solution is reduced, first to the corresponding dark green hydroxylamine derivative, and finally, to the amine derivative, which has an intense red colour(1). The best results were obtained with 1·5-nitro-anthraquinone sulphonic acid(2). The 1·6 and 1·8 derivatives were found to be less suitable. The test is a delicate one. About 0·1 mgm. of glucose per 100 c.c. can be detected on comparison with a blank test with the reagents.

The technique embodied in the following directions was ultimately adopted. Prepare a protein free filtrate from blood by Folin's tungstate method. Dilution is consequently 1 in 10. Measure 5 c.c. of this filtrate into a 15 c.c. graduated test tube. Measure with a normal pipette graduated in ·02 c.c., or with a microburette 1·5, 1·8, 2·1, 2·5 and 3·0 c.c. of ·02 p.c. glucose into 15 c.c. graduated test tubes numbered 1 to 5. These quantities are in a geometrical series having an approximate common ratio of 1·2, and correspond to 0·06, 0·072, 0·084, 0·1, and 0·12 p.c. of sugar in the undiluted blood with the filtrate of which they may be found to match in colour. Add to the contents of each test tube 1·0 c.c. of

0.1 p.c. aqueous solution of 1.5-nitro-anthraquinone sulphonic acid and 2.0 c.c. of 50 p.c. potassium carbonate. Dilute to 10 c.c. with distilled water measured from a burette, and mix thoroughly. Part of the sparingly soluble potassium salt of the sulphonic acid separates as a crystalline precipitate, which dissolves on heating. Immerse the six test tubes in boiling water for 8 to 10 minutes. Cool thoroughly, make up each of the solutions to 12 c.c., and compare the tint of the tube containing blood filtrate with those of the sugar solutions. An approximate estimate of the blood sugar is thus obtained. Finally, compare in the colorimeter the blood filtrate and the solution of sugar, which most nearly matches that derived from the blood filtrate. When blood from cases of suspected hyperglycæmia has to be examined, half the quantity of blood filtrate may be taken for the estimation, and allowance made for this fact in the calculation.

A large number of estimations of the sugar in blood derived from normal human cases, from animals with hypoglycæmia following the injection of insulin, and from animals with hyperglycæmia have been made, and the sugar in each sample of blood has also been estimated by Folin and Wu's most recent method. The new method gives results about 10 p.c. higher than those obtained by Folin and Wu's method.

(1) L. Wacker. *Berichte d. deutsch. chem. Gesellschaft*. 35. 666. 1902.

(2) R. E. Schmidt. *Ibid.* 37. 71. 1904.

### **The physical theory of phagocytosis.** By A. G. McKENDRICK.

When an assemblage of leucocytes operates on an assemblage of particles, and ingestion occurs, it is well known that at any subsequent instant of time the numbers of leucocytes which contain 0, 1, 2, ... particles are denoted by the successive terms of a binomial series, the index of which, so far as experience goes, is invariably a negative quantity.

In 1913 the author, in considering the case of leucocytes operating in a discrete assemblage of particles, found that theoretically this negative index indicated that the appetite of the leucocyte increased with the number of previous ingestions. This unsatisfying result led him to introduce the secondary assumption that the particles did not remain discrete, but agglutinated during the course of the experiment into clumps of varying size, which were ingested as such. It appears that with this additional assumption the form of the mathematical solution, in the

case where leucocytes and particles are initially discrete, is unaltered; that the index of the binomial must invariably be negative; and that its numerical value indicates the ratio between phagocytic and agglutinative avidities.

The theory—arrived at independently—is found to include that of Schmoloehowsky regarding the coagulation of colloids, and may be considered as an extension of that theory to phagocytosis.

# PROCEEDINGS

## OF THE

# PHYSIOLOGICAL SOCIETY,

### *June 13, 1925.*

**Effects of section of the vagi nerves on the motor activity of the stomach.** By E. D. MCCREA, B. A. MCSWINEY and J. S. B. STOPFORD.

In order to observe the effects of section of the vagi upon the motor function of the stomach, the nerves were resected as they lie upon the pars abdominalis of the œsophagus. If one nerve only was to be divided this was performed in the neck. The animals used were the rabbit, cat and dog, and the results were studied by X-ray examination.

Care was taken to establish the normal variation in each individual animal by repeated X-ray examinations before operation, and precautions were taken, as far as possible, to exclude psychic effects. Post-mortems were carried out on those animals which were killed or died, the stomach examined and the nerve sections verified. Observations after operation have been carried out during periods extending up to six months.

Number	Dilatation	Contractions		Emptying	
		Depth of	Interval between	Initial	Total
Bilateral Vagotomy 15	None detected	Increased	No change detected	Marked decrease	Decrease
Unilateral Vagotomy 11	No variations from within the normal limits observed				

The accompanying table shows the results which we have obtained up to the present. It is at once obvious that we differ on several points from other investigators, and especially on the question of emptying time.

To sum up our findings; unilateral vagotomy is without effect upon the stomach, and in this we agree with almost all observers. The results of bilateral vagotomy may be divided into two groups: those which are

temporary or which are compensated for in time, and those which may be considered permanent. The first group comprises paresis and dilatation, the second includes increased depth of peristalsis, possibly some slight dilatation and a marked decrease in the initial, with a slight decrease (about one hour) in the total emptying times. Of these, the early initial emptying is the most prominent feature, the food appearing to pour into the duodenum, often unassociated, at least at first with peristalsis. This appears to be due to a semi-patulous condition of the pyloric sphincter.

Possible explanations of the discrepancies on the question of emptying time between our work and that of other observers are: first, that if the period of observation is confined to the period during which temporary paresis and dilatation occur a delayed total emptying time may be noted; and secondly, that if the nerve supply of the pylorus escapes(1), as it is liable to do unless care is taken, dilatation and retention will occur.

(1) McCrea. *Journ. of Anat.* 59. 17. 1924.

The expenses of this investigation have been defrayed by a grant from the Government Grants Committee of the Royal Society.

### **The magnesium content of cerebrospinal and other body-fluids.**

By HENRY COHEN.

Observations on 15 cases show that the percentage of Mg in cerebrospinal fluid, in normal and pathological states other than meningitis, is constantly greater than that of the contemporary blood serum. In pleural and peritoneal effusions it is, on the other hand, smaller than in the serum, although the chloride concentrations in these effusions, as also in normal cerebrospinal fluid, are always greater than in serum. In meningitis (three cases) the excess of Mg in the spinal fluid becomes less marked.

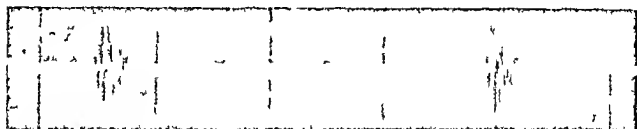
There is a considerable post-mortem increase in the Mg content both of blood-serum and of spinal fluid, and that of the serum soon becomes greater than that of the spinal fluid.

Oral administration of 20-30 grams of magnesium sulphate has no effect on the Mg content of blood-serum. Intramuscular injection of 1.8-2 grams is rapidly followed by an increase in the Mg of serum, reaching a maximum (40-100 p.c. increase) in 2-2½ hours.



**Phono-arteriograms.** By J CRIGHTON BRAMWELL  
and SYLVIA K HICKSON<sup>1</sup>.

Optical records of the arterial sounds were obtained by Wiggers and Korns, but, so far as we are aware, this research was discontinued, and we have not been able to find any reference to the subject in the literature. Some recent observations made by one of us, on the form of the pulse wave, suggested that the modification produced in the arterial sounds by the application of a compressing armlet was intimately connected with the change in form which the pulse wave undergoes in passing



through the compressed segment. We are at present studying this problem, and for the purpose of recording the arterial sounds we have adopted the optical technique employed by Wiggers for recording heart sounds, namely, a Frank's segment capsule with a very thin membrane freshly made from rubber solution. An open side tube allows free communication between the system and the outer air. The membrane has a high natural frequency, and its ability to follow rapid vibrations has been tested by means of tuning forks. The figure shows two pulse cycles recorded over the brachial artery, immediately below a pneumatic armlet in which the pressure was maintained at 70 mm Hg. Time intervals 0.2 secs.

**Gas diffusion through the frog's lung.** By G LILJESTRAND  
and A V SÄHLSTRÖM

With varying filling of the lung it is to be expected, as can easily be shown, that the diffusion should be in proportion to  $V^{\frac{2}{3}}$ , if  $V$  is the volume of gas in the lung. In experiments with isolated and surviving frog's lungs, where pure  $\text{CO}_2$  inside diffused into air or  $\text{O}_2$  outside, good agreement was found between the observed and calculated values.

Variations of temperature between 4° C and 39° C give for  $\text{CO}_2$

<sup>1</sup> Working for the Medical Research Council

hardly any difference in the diffusion. For acetylene the diffusion was found to rise nearly, 60 %, when the temperature rose from 4° C. to 37° C. According to Hufner a considerable decrease for both gases would have been expected with the increase of temperature.

CO<sub>2</sub> was found to diffuse through the lung at room temperature about 40 times as rapidly as O<sub>2</sub> and about 80 times as rapidly as N<sub>2</sub>.

### **Action of pilocarpine on surviving strips of the stomach.**

By G. L. BROWN and B. A. McSWINEY.

Investigations have been carried out on the action of pilocarpine on strips taken from the cardia, fundus, upper and lower body, antrum and pylorus of the stomach of the rabbit, cat and dog, and in the cat from the

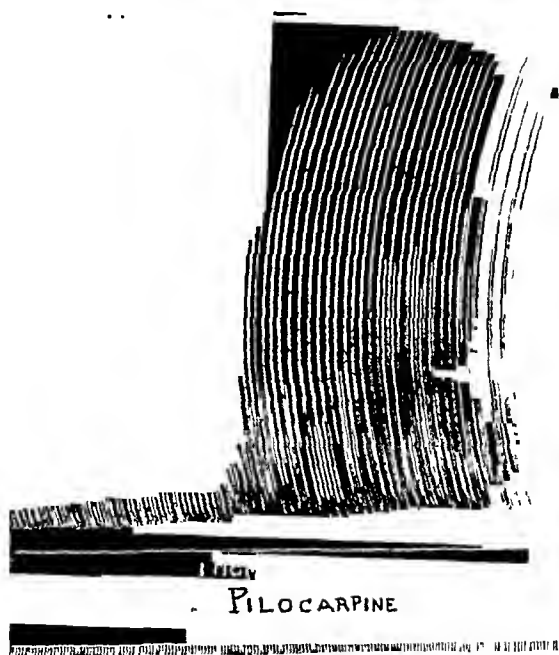


Fig. 1. Action of Pilocarpine on antrum of cat's stomach. Tracing to show augmentation of rhythmic contractions. Time intervals - 30 seconds.

sling of Forssell. The movements were recorded by suspending the strips in oxygenated Tyrode's solution in the intestine chamber previously described (1). An aluminium recording lever with a twisted thread suspension was employed. Spontaneous rhythmic contractions were recorded from the preparations made from the different regions

with the exception of the cardia. Pilocarpine in a dilution of 1 in 250,000 brings about a permanent shortening of the muscle fibres in the upper region of the stomach: the effect becomes successively smaller till in strips taken from the pyloric region the base line maintains a constant level.

*Action of pilocarpine.*

Region	Rise in base line	Rhythmic movement
Cardia	+++	0
Fundus	+++	+ or -
Upper body	++	+
Lower body	+	++
Pyloric antrum	0	+++
Pyloric sphincter	0	+
Sling of Forssell	+++	++

Augmentation of rhythmic movement is most pronounced in the lower regions of the stomach, decreasing progressively to the fundus: in the latter region contraction may be diminished. The sling of Forssell

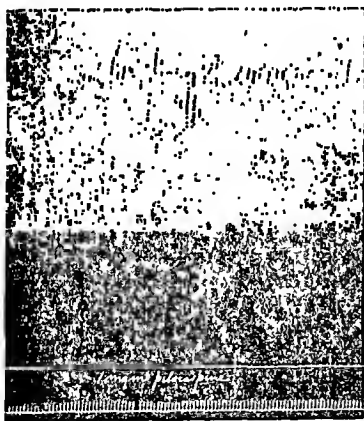


Fig. 2. Action of Tracing to show contractions. T. : . . . : stomach. rhythmic

occupies an intermediate position: augmentation of movement is well shown together with a rise of base line, which is not distinguishable from the reaction of the fundic region.

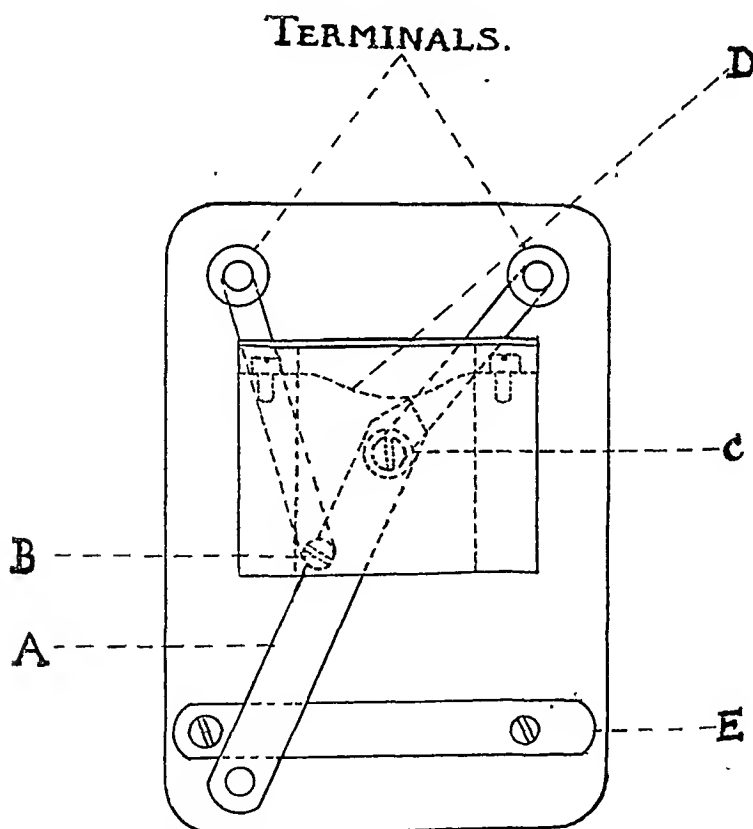
The sphincters of the stomach behave in an antagonistic manner. The drug causes in the cardia a rise in base line, but rhythmic movement does not occur. In the region of the pyloric sphincter there is no permanent change in the length of the muscle fibres, while augmentation of movement is easily elicited.

(1) McSwiney, B. A. *This Journal, Proc. Physiol. Soc.* lvi. 1922.

The expenses of this investigation have been defrayed by a grant from the Government Grants Committee of the Royal Society.

### A simple make-and-break key. By B. A. McSWINEY.

A simple make-and-break key has been devised to give instantaneous make-and-break shocks.



Make-and-break key (two-thirds actual size).

The key is mounted on a thick fibre block. A brass arm *A* swivels about a brass bush screw *C*. One end of the arm *A* is fashioned into a cam which presses against a piece of steel spring *D* attached to two fibre

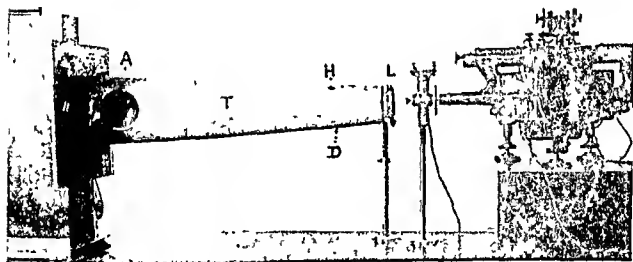
uprights. In the "On" position the arm *A* makes contact with the stud *B*. The brass strip *E* prevents rocking of the arm. The two terminals are connected with the stud *B* and the bush screw *C*. The key is covered by a brass casing screwed to the two fibre uprights.

The key has the advantage that it gives instantaneous make-and-break shocks and is not affected by vibration. The key is suitable for class work as it is unbreakable.

I am indebted to Mr F. S. Wilson of the Physiology Dept., University of Manchester, for assistance in designing and constructing this key.

### A camera shade. By J. CRIGHTON BRAMWELL<sup>1</sup>.

When taking photographic records either for clinical or for experimental purposes it is sometimes convenient to work in full daylight; and with this object in view, a screen has been designed to shade the front of the camera. It consists of a funnel-shaped tube (*T*) made of stout cardboard. This is shown in the illustration, stretching from the cylin-



drical condensing lens (*L*) (fitted to the standard Cambridge Electrocardiographic Outfit) to the front of the camera, to which it is attached by four hooks on a light wooden frame. To focus the shadow of the string, or to follow its movements, the observer looks through a small hole (*H*) near the proximal end of the tube, at an image of the front of the camera, formed by a small plane mirror. The mirror is mounted on a universal

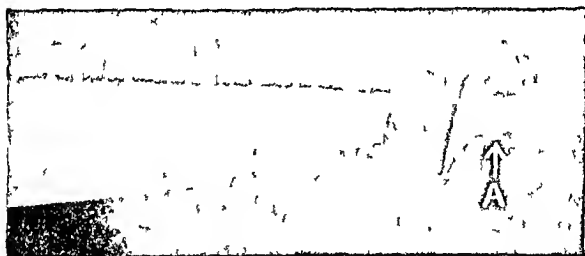
<sup>1</sup> Working for the Medical Research Council

joint, so that it can be set at any desired angle by means of a handle (*D*) which projects through the wall of the tube. Near the distal end of the tube is an aperture (*A*) large enough to admit the observer's hand, for the purpose of adjusting the shutter of the plate camera. During an exposure this aperture is covered by a curtain of black cloth. Extraneous rays may be prevented from impinging on the cylindrical lens by a flat cardboard shield, placed behind the time-marker, in a plane at right angles to that of the axis of the funnel.

PROCEEDINGS  
OF THE  
PHYSIOLOGICAL SOCIETY,  
*July 4, 1925.*

**A new rhythm in striped muscle.** (*Preliminary communication*)  
By H S D. GARVEN

If a slow series of stimuli (one or two per second) are given to the sciatic nerve of a frog and the contractions of the gastrocnemius recorded on a slow drum, the base-line drawn out by the lower ends of the recorded contractions may show a marked rise with the onset of fatigue. In some cases this degree of contracture is marked, in others it is completely absent. So far the factors controlling this presence or absence of contracture have not been fully worked out. Only where this contracture is present has the rhythm shown itself.



In the rising base-line as a rule no rhythm can be detected, although in one case it has been slightly in evidence. If however the stimuli are stopped during the development of the contracture for one minute and then restarted, the base-line, now traced out as it rises rapidly to or above its previous height, shows a very marked undulation (see Fig. A). The upper points of the contraction records do not always show this undulation but often do. The first wave is the most marked and after three or four progressively smaller waves the base line remains at a constant height or rises evenly.

Prima facie it is obvious that contracture must be present. The rhythm has been found both before and after section of the sciatic nerve, and experiments on stimulation of the nerve roots above the junction of the rami communicantes are now being made. So far it is impossible to make any statement on the influence of the sympathetic fibres. The resemblance between these tracings and those of Botazzi, showing the two rhythms of the tortoise heart, is very striking.

Experiments are in progress to determine the exact conditions under which the rhythm appears.

**Apparatus for measuring variations in the velocity and temperature of air currents.** By H. M. VERNON and J. J. MANLEY.

It is generally supposed that variable air currents create a greater sensation of air movement than steady currents, though no definite proof of this hypothesis has been adduced. In order to obtain evidence on the point, we have designed a *hot-wire anemometer*. The effects of air currents on the electrical resistance of a heated wire have been investigated by Ser, Kennelly, and King, whilst J. T. Morris devised a portable hot-wire apparatus for measuring air velocity. In all the forms of apparatus used the temperature of the heated wire was kept constant by varying the strength of the current, but we have employed a constant current (0.5 amp.) and a variable resistance, and have thereby obtained an instrument which indicates quick changes of velocity. The apparatus consists of a "detector" composed of three platinum wires (17 mm. long and 0.08 mm. in diameter), fixed in three planes at right angles, and supported on an ebonite ring, so as to catch air currents moving in all directions. The wires were heated to about 130° C. by the current from an accumulator, and they form one arm of a Wheatstone bridge, the other arms of which are composed of "Eureka" wire (which has no temperature coefficient). One of these resistances is adjustable, whereby the resistance of the hot wire can be balanced. Connected with the bridge is a Paul unipivot dead-beat galvanometer of 9.5 ohms resistance, and a shunt. It was read at  $2\frac{1}{2}$  second intervals synchronous with the ticks of a metronome. The apparatus was calibrated by placing the detector in a small wind tunnel, the air velocity in which was determined by means of a kata-thermometer. To correct for possible differences in velocity at various parts of the tunnel, the determinations were always repeated after exchanging the position of the instruments. It was found that at deflections corresponding to an



an velocity of over 60 ft per minute the galvanometer deflection varied as the cube root of the air velocity, but at lower velocities the relationship did not hold, and the calibrations had to be deduced from the curves. Series of observations were made at  $10^{\circ}$  and  $24^{\circ}$  C, so as to determine the temperature effect

The variability of air currents relates to temperature as well as velocity, and in order to measure the temperature variations we designed a *thermopile* consisting of 20 junctions of iron and nickel wire. These junctions were hammered out very thin, and the wires were fixed in a boxwood ring so as to catch air currents from all directions. The ring could be pushed down so as to fit tightly in an annular trough, which prevented access of air to the lower junctions. The thermopile was connected with a second galvanometer similar to that above mentioned, and was read by a second observer synchronously with the other instrument. It was calibrated by putting the junctions in jets of water differing slightly in temperature



The figure shows two sample pairs of records. In (a) the oscillations of air velocity and temperature are somewhat larger than the average. It will be seen that they usually synchronise, rise and fall of velocity (due chiefly to draughts of cool air from open windows) being accompanied by fall and rise of temperature. In (b) the oscillations are smaller than usual, and do not synchronise.

Observations were made both in summer and winter at twelve factories, when the sensations of air movement experienced were recorded (according as the air felt 'very stagnant,' 'very fresh,' or one of the seven intermediate classes), and the oscillations of velocity and temperature were measured. They were calculated as a mean variation on their

respective averages, and it was found that the greater the oscillations of velocity the greater the sensation of air movement. The correlation ratio of the mean variation of air velocity on air sensation was  $\cdot 365$  to  $\cdot 418$ . No clear proof was obtained that the small oscillations of temperature experienced had any effect.

**The influence of injections of emulsions of testes and prostate and of testicular extracts upon the nitrogen metabolism of normal and thyroidectomised rabbits.** By V. KORENCHEVSKY and M. CARR.

From our previous work (*Brit. Journ. Exper. Path.* 6, 74, 1925) we found that testis emulsion, similarly to that of kidney, when injected subcutaneously into rabbits, slightly decreased the nitrogen metabolism during the period of injections and in most cases during the succeeding 6—9 days. This effect of testis emulsion cannot be considered a specific one because of the similar results obtained with kidney emulsion.

An emulsion of prostate alone or in combination with testis when injected increased the nitrogen metabolism up to 11 per cent. particularly during the two or three days following the period of the injections.

The above-mentioned effect of testis emulsion was contrary to expectation because castration in rabbits produces a fall in the nitrogen metabolism (Korenchevsky(1), 1925). Therefore, we were inclined to explain these contradictory results by the possible presence in the rabbit's testis of two different principles, one increasing, the other decreasing nitrogen metabolism, the latter prevailing when testis is injected as an emulsion. The following experiments seem to justify this theory.

Ashby(2) (1923), Baker, Dickens and Dodds(3) (1924) and several others have discovered insulin or an insulin-like substance in different tissues, kidney included. Mr F. H. Carr, at the British Drug Houses laboratories, isolated a similar substance from bull testes, which we have used experimentally. When about 0.8—1.3 mg. per kilo and diem of this substance were injected subcutaneously into normal or castrated rabbits, only a slight fall, if any, in the nitrogen metabolism, was produced. Similar or even smaller doses injected into thyroidectomised rabbits produced a much more pronounced decrease of the nitrogen metabolism (see table).

An emulsion of testis also decreased the nitrogen metabolism of thyroidectomised rabbits much more than that of normal or castrated animals.

This similarity between the influence upon nitrogen metabolism of testis emulsion and that of insulin suggest that the fall in the nitrogen metabolism obtained in rabbits after the injection of testis emulsion might be partly due to the presence of insulin in the testes. The same explanation might apply to the decrease in the nitrogen metabolism of rabbits after injection of kidney emulsion.

Experiments with prostate emulsion injected into normal and thyroidectomised rabbits have shown that the thyroid gland must be present in order to obtain an increase in the nitrogen metabolism: in thyroidectomised rabbits an emulsion of prostate alone produced practically no change in the nitrogen metabolism, whereas an emulsion of both prostate and testis usually decreased the nitrogen metabolism, although to a much less degree than when an emulsion of testis alone was injected.

TABLE

Percentage change from the normal in the N output in the urine of rabbits after injection of insulin like extract from testis, and of different emulsions.

Injected	Number of Expts	Thyroids present (+) or removed (-)	Experimental periods.			
			I Inject period 3 days	II Contr period 2 days	III Contr period 3 days	IV. Contr. period 2 days
Kidney emulsion	3	+	-4.1	-1.9	-1.8	
Testis emulsion	1	+	-1.6	-2.5	-2.4	
	3	-	+0.2	-12.1	-7.1	-7.9
Prostate emulsion	1	+	+3.1	+9.0	+1.5	+3.3
	3	-	+0.5	-0.2	-0.5	-1.6
Prostate and testis emulsion	5	+	+0.5	+9.3	+3.3	+1.3
	3	-	-2.1	-3.3	-0.3	-1.7
Testis insulin						
About 0.8 mg. per chem and kilo	1	+	-3.2	-1.8	-4.3	
	2		0	-8.6	-9.7	
About 1.3 mg. per chem and kilo	3	+	-1.2	-0.8		
	3	-	-17.5	-26.9		

Kidney or prostate emulsion had no pronounced or constant influence upon the excretion of urine in normal or castrated rabbits. Testes emulsion and in most cases testicular insulin quite definitely decreased diuresis. This effect produced by testes emulsion and by insulin was not changed by thyroidectomy, but when prostate emulsion was injected into thyroidectomised rabbits a definite increase of diuresis was observed. Therefore it is possible that insulin-like substances and others present in testes and prostate may influence the excretion of urine.

(1) Korenchevsky, V. Brit J Ex. Path. 6 21. 1925.

(2) Ashby, J. Amer J. Physiol. 67 77. 1923.

(3) Baker, S., Dickens, F and Dodds, E. Brit. J. Ex. Path. 5. 327. 1924.

**Action currents in sensory nerve fibres.** (*Preliminary communication.*) By E. D. ADRIAN and SYBIL COOPER.

Using a three-valve resistance-capacity coupled amplifier in conjunction with the capillary electrometer, we have found it possible to obtain very clear records of the action currents occurring in sensory nerve fibres in response to 'natural' stimulation of the end organs. We are greatly indebted to Prof. Gasser for supplying us with details of the amplifier used by him. This has been modified in certain details and the present apparatus gives an amplification of 1500—2000 with an extremely steady base line. If leads are taken from the sciatic of an isolated sciatic-gastrocnemius preparation of the frog, a succession of action currents appear in the nerve whenever the muscle is extended by a weight (cf. Forbes(1) and de Meyer(2)). The action currents recur at irregular intervals; the maximum frequency we have recorded is in the region of 400 a second, though the frequency in each nerve fibre is probably lower. If a 50 gram weight is hung on the muscle the succession of action currents continues with diminishing frequency for two or three minutes and occasional impulses occur for ten minutes or more. From the size of the action currents we conclude that most of them represent the activity of a very small number of nerve fibres.

A rapid succession of action currents of the same type can be led off the frog's sciatic (isolated from the spinal cord) when the skin of the foot is pinched with forceps. If leads are taken from the internal saphenous nerve of the cat (isolated from the cord) the record shows a succession of action currents even in the absence of stimulation and these increase in size and frequency when the skin is pinched or pricked.

We have used a number of different controls to satisfy ourselves that the oscillations recorded are true action currents accompanying nervous impulses. Their time relations (from the analysis of isolated diphasic and monophasic responses) do not differ greatly from those of the action currents set up by electrical stimulation of the nerve trunk and there is no obvious difference in the time relations of the action currents due to pricking the skin and those due to stretching a muscle.

If leads are taken from a motor nerve (the peroneal of the spinal cat or the frog's sciatic) in connection with the spinal cord, and the animal is stimulated by pinching the foot, etc., an irregular succession of oscillations appear in the nerve at frequencies varying between 50 and 300 a second. The time relations of each oscillation (monophasic) are on the whole longer than those in the sensory nerve, as would be expected if

each oscillation were due to a discharge of impulses not completely in phase with one another (cf Forbes and Giegge<sup>(3)</sup>) Our results as to the frequency of the oscillations are in agreement with Gassei and Nowcomer's<sup>(4)</sup> observations on the phrenic nerve A full account will be published shortly

(1) Forbes, Campbell and Williams Amer Journ 69 283 1924

(2) J. de Meyer Arch Internat de Physiol 16 61 1921

(3) Forbes and Gregg Amer Journ 37 148 1915

(4) Gassor and Nowcomer Ibid 57 1 1921

**Respiratory exchange during anaesthesia.** (*Preliminary communication*) By D F KANAAR, M S PEMBREY and N H SKELTON BROWN

In order to investigate the effects of anaesthesia upon the metabolism of the body a series of determinations of the respiratory exchange of rabbits and rats have been made, in some of the experiments the urine has been examined for glucosuria and estimations of the percentage of glucosuria in the blood have been made In the table examples are given to compare the effects of various drugs with those produced by lack of oxygen due to poisoning with pure carbon monoxide The weights represent grammes and the periods are for one hour unless it is stated otherwise

A rise in the percentage of sugar in the blood and glycosuria appear to be associated with the low respiratory quotients, but further experiments are necessary and are in progress, especially in relation to the nutrition of the animal

H <sub>2</sub> O	CO <sub>2</sub>	O <sub>2</sub>	$\frac{CO_2}{O_2}$	Remarks
1.02	2.35	1.73	0.99	Rabbit wt 2160 grms Normal R T 38.8° Average of 3 hours
0.73	1.94	1.47	0.96	" 30 mins after 15 c.c. of 25% urethane R T 36°
				Average of 2 hours
0.85	1.88	1.78	0.77	18 hours later R T 37° Average of 4 hours
0.90	1.52	1.28	0.87	Rabbit wt 1627 grms Normal R T 39°
0.60	1.23	1.16	0.77	36 mins after 1 gm chloral R T 35°
0.73	2.17	1.82	0.86	1½ hours later recovering R T 38°
1.36	2.25	1.85	0.89	Rabbit wt 2174 grms. Normal
1.02	1.80			during ether anaesthesia R T 37.5°
0.70	1.35	1.01	0.97	Young Rabbit wt 535 grms Normal R T 39.2°
1.05	0.65	0.73	0.62	" 3 days later carbon monoxide poisoning
				R T 33°
0.73	0.87	0.70	0.90	Rabbit wt 5635 grms Normal
0.62	0.78	0.62	0.92	½ period going under CO
0.74	0.86	0.48	1.30	under CO
0.71	0.95	0.66	1.05	" CO shut off
0.85	0.91	0.80	0.83	" average of 3 hours of recovery
0.60	0.42	0.48	0.63	Black and White Rat ♂ under ethylene
0.53	0.53	0.48	0.80	" " " recovery

} Periods of 1½ hours

**Effects of pituitrin in man. (*Preliminary communication.*)**

By R. S. AITKEN and J. G. PRIESTLEY.

In view of the remarkable effect of pituitrin injections (1 c.c. B. W. & Co.) in delaying for 5 to 6 hours the diuresis which normally follows water drinking, we decided to investigate other results of such injections.

We have injected 1 or 2 c.c. intramuscularly and have noted the following effects:

*Circulatory changes.* The most striking effect is the onset of pallor which has been described by Lawrence and Hewlett(1). We have found that it comes on within 5 to 10 minutes and begins to pass off in about an hour, but it is difficult to be sure when the colour is completely restored.

The capillary contraction due to pituitrin does not seem to prevent in any way the dilation of capillaries caused by gentle stroking of the skin.

An armlet was applied and pumped up to a pressure of 80 mm. Hg at 4.28, 1 c.c. of pituitrin having been injected at 4.26. No change was noted in the colour of the congested arm by 4.50, though the usual pallor was noted in other parts. The armlet pressure was then reduced to 60 mm., and it was found that the vessels rapidly filled up again when emptied by stroking. Pressure reduced to 10 mm. at 5.4; very slight congestion of the arm. The contraction of the capillaries brought about by pituitrin does not therefore seem to be forcible enough to overcome a pressure of much more than 10 mm. Hg.

The pulse rate increases by about 20 beats per min. within 20 min. of the injection and returns to normal within an hour. Systolic arterial pressure shows a rise of 5 mm. or so only, which begins within 5 min. of the injection and passes off within 15 min. The diastolic pressure seems to be hardly altered.

*Circulation rate.* We have made several attempts to measure the circulation rate by the method of Douglas and Haldane using the arterial and venous CO<sub>2</sub> as index. We find that both arterial and venous CO<sub>2</sub> pressures are slightly diminished about 20 min. after the injection, returning to normal within about 2 hours. The results seem to indicate a very slight increase in the circulation rate, but cannot be regarded as reliable in view of the respiratory changes noted below and the fact that alveolar air changes seem to be very transitory. We propose to continue these observations and to determine the venous oxygen pressure if possible.

*Respiratory changes* The rate of respiration shows a somewhat inconstant increase. In one case it increased from 15 to 21 per min within 5 min of the injection, falling to 11 per min within 30 min and returning to 15 within 80 min.

The total metabolism shows an increased removal of  $\text{CO}_2$  and diminished absorption of oxygen for a short time after pituitrin. Thus

Time	$\text{CO}_2$ expelled	$\text{O}_2$ absorbed	R.Q.	
1 15	182.9			
2 5	183.0	221.3	83	
2 51	166.6	228.6	82	
3 11				1 cc pituitrin injected
3 28	221.9	198.3	1.12	
3 41	192.9	231.5	83	
3 51	163.4	270.6	61	
4 31	177.8	221.8	80	

These results are difficult to interpret as we have been unable to find a corresponding change in the alveolar  $\text{CO}_2$ . If such a change occurs it is probably very transitory.

On the whole it seems probable that the effect of pituitrin is to cause capillary contraction with consequent diminished absorption of oxygen and anoxæmia, but further and more numerous observations on the alveolar air are required.

The effects of pituitrin on the circulation and respiration, however, seem to be too transitory to explain the prolonged inhibition of diuresis.

(1) Lawrence and Hewlett. B. M. J. 1 938 1925.

### Certain difficulties which arise in determining the rate of diffusion of carbon dioxide in alkaline solutions. By B. J. CORRINGWOOD and D. LEVI.

In the course of experiments on the diffusion of dissolved and combined  $\text{CO}_2$  in tap water and in alkaline solutions evidence appeared that gravity had a distinct influence on the rate of diffusion.

I. A solution of 0.21%  $\text{NaHCO}_3$  with phenol red as an indicator was saturated with alveolar air. The solution showed the intermediate tint of a  $p_{\text{H}}$  approximating to that of blood. A number of tubes with a diameter of 5 mm. were filled with this solution. The tubes were arranged on a vertical board like the spokes of a wheel, the bottoms of the tubes being at the centre of the wheel. After 24 hours exposure to room air the following changes were noted.

In the inverted vertical tube a narrow band of red appeared at the lower end. On revolving the tube so that this lower end became uppermost the red band sank *en masse* into the fluid below.

In the non-inverted vertical tube a diffuse pink extended some distance down the tube and occupied an obviously greater area than the red band in the first tube.

In the tubes occupying intermediate positions there occurred conditions intermediate between the first and second tube in a regular sequence.

To exclude the possibility of the evaporation of water from the surface of the tubes being responsible for their differences similar experiments were conducted in air saturated with water vapour. The results were the same as before.

II. Two tubes similar to the above were filled with tap water to which phenol red was added as an indicator. A pink colour resulted. These tubes were placed in a vessel containing alveolar air, both of them being in a vertical position, and one of them being inverted. After 24 hours the following changes were noted.

In the inverted vertical tube a narrow band of yellow appeared at the lower end. On revolving the tube so that the lower end became uppermost the yellow band sank *en masse* into the fluid below.

In the non-inverted vertical tube a diffuse yellow extended some distance down the tube and occupied an obviously greater area than the yellow band in the first tube.

That identical results of this nature should follow both the exit and the entry of  $\text{CO}_2$  is not easy to explain. Yet whatever the explanation may be, such phenomena as the above render an enquiry into the diffusion of  $\text{CO}_2$  a matter of some difficulty and complexity.

The matter is being further investigated in the hope of elucidating the physical or chemical factors involved.

#### **Excretion of water by the kidneys.** By O. A. BEADLE and J. G. PRIESTLEY.

In 1916 one of us published<sup>(1)</sup> some experiments on the effect on urinary secretion of drinking a salt solution made up to represent the salts of blood plasma, *i.e.* 0.62 % NaCl and corresponding amounts of the other ions of the plasma. Drinking 3 litres of this solution resulted in a diuresis attaining a maximum of about 1 litre per hour.

We have continued this work by comparing the results of drinking more concentrated solutions with the diuresis which follows the drinking of tap water.

A solution was made up containing 0.86 % NaCl, 0.45 %  $\text{NaHCO}_3$



and corresponding small amounts of Mg, Ca and K salts, *i.e.* 0.16 N as regards Cl

Two litres of this were drunk and the consequent excretion of urine was about 40 to 50 c.c. per hour as contrasted with 40 to 20 c.c. per hour when nothing was taken, and 630 c.c. per hour when water was drunk. Drinking 2 litres of the salt solution diluted with water to 9, 85 and .8 the original strength *i.e.* Cl = 144, 136 and 128 N, was followed by urinary excretion of 87 falling to 30, 90 falling to 50 and 90 falling to 70 c.c. per hour. These volumes are in marked contrast with the diuresis following water and the first salt solution, which was .116 M as compared with 216 to 174 M for the dilutions of the second solution, *t*. These results confirm and extend those of Adolph (2) (3).

We conclude, therefore, the stimulus to the kidney to excrete water is not the concentration of water in the plasma, but the diffusion pressure, *i.e.* the ratio of water molecules to total molecules, and that a very small change in this ratio is an effective stimulus. It is also evident that a solution which has the same concentration of electrolytes as the plasma acts as an effective stimulus to the kidney to excrete water because, owing to the pressure of protein in plasma, the ratio of water molecules to total molecules is different in the two cases. The reaction of the kidneys is a delicate measure of the effective volume of the plasma proteins.

(1) Priestley *J. Physiol.* 50: 304, 1916.

(2) Adolph *J. Physiol.* 55: 114, 1921.

(3) Adolph *Amer. J. Physiol.* 63: 432, 1923.



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